The exercise physiology of snapper (*Pagrus auratus*): Implications for the better commercial harvesting of an iconic New Zealand finfish

Sarah Elizabeth Coxon

A thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy in Zoology

University of Canterbury

2014

TABLE OF CONTENTS

LIST OF FIGURES

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

Chapter 7

LIST OF TABLES

Chapter 2

Chapter 3

Chapter 4

Chapter 6

Chapter 7

ACKNOWLEDGEMENTS

The last few years have certainly been one heck of a rollercoaster; from earthquakes to equipment malfunction, two dream-fulfilling trips to Antarctica as a "research assistant," to the never-ending phase that was the writing of this thesis, it's a wonder the thesis ever got finished! But here we are, and my sincere thanks go out to all of those whose assistance, enthusiasm and encouragement were instrumental in making it happen.

First and foremost, a huge thankyou to my supervisor, Professor Bill Davison, for his support, advice and enthusiasm throughout this project. I couldn't have asked for a better supervisor to help me through the minefield that (I now know) is a PhD. You always found time in your busy schedule to read drafts, discuss my "quick questions" and to listen to me freak out about almost everything. Your willingness to share your wealth of knowledge and experience was very much appreciated, and your passion for science, inspiring.

To my assistant supervisors, Alistair Jerrett and Malcolm Forster, I wish to extend my gratitude for the valuable advice, stimulating discussions and encouragement.

I would like to extend a huge thankyou to the amazing technical support team at UC; in particular, Gavin Robinson, for always being so willing to help with tasks big and small, despite his own insane workload, and to Alan Woods, for his dedication to the refurbishment of the swimming flume and the automation of the respirometry systems, both real game changers! Thanks also to Jan McKenzie, Renny Bishop, Nick Etheridge and Matt Walters.

To the team at Plant and Food Research – Suzy Black, Nick Tuckey, Jed Janssen, Sharon Ford and Len Forgan – thankyou all for your willingness to offer assistance and advice, and for making me feel like a part of the team (even from far away). Special thanks also for collecting the samples from trawl-caught snapper on my behalf, which made Chapter 6 possible.

To the UC physiology students, past and present - you guys rock! Esme, Rachel, Charlotte, Rathi, Mauricio, Ash and Jacqui – it made it so much easier to know that we were all facing similar challenges, and it wasn't just me! Seriously though, your feedback and ideas on all manner of things fish-related was much appreciated.

To the girls of "The Fort" – Charlotte, Rachel and Olivia. Never was there a more rocking, awesome office to work in (and with its own pirate-themed Facebook page, no less!). It would only be fitting to celebrate with a Fort Field Trip of some description.

None of this would have been possible without the love and support of my family: Mum, Dad, Nana, Grandma, and Grandad Bill and Grandad Jack (both dearly missed). You have all been instrumental in supporting and encouraging me over my years of study, and inspire me to be the best that I can be. Also, to the best-ever siblings, Amanda and Matthew – you guys are tops. Oh, and Matt, remember when you said you'd put on the "mad shout" when I finally finished……

Finally, to my partner Sam – you are my rock. This project was as much yours as it was mine, as you lived through the highs and lows with me, and endured my never-ending yarns about fish like a trooper. Your support and quiet encouragement, and ultimately your patience (!) were invaluable in seeing me through.

I also wish to acknowledge the financial support the New Zealand Institute of Plant and Food through their Higher Value Seafoods division, and the of University of Canterbury (Brownlie PhD scholarship).

ABSTRACT

Worldwide, an increasing demand for fish and fisheries products, together with socioeconomic pressure for industry expansion, is placing considerable pressure on wild fish stocks – more than 80% of which are considered by the Food and Agriculture Organisation of the United Nations (FAO) to be either maximally- or over-exploited. Adding value to the existing catch and/or improving the sustainability of current wild capture methods may offer a means of providing industry growth while negating the need for increased landings. In particular, the peri-mortem condition of a fish plays an integral role in the condition of the tissues post-mortem and hence in product quality, with harvesting techniques that result in stress or fatigue yielding a lower quality product. An understanding of the physiology of the target species and its response to harvest is therefore essential to implementing targeted improvements in harvesting technologies. For species harvested using trawl-based technologies, this includes knowledge of their exercise physiology, in particular their swimming capacity, since this is a key determinant of the interaction between fish and trawl gears, and hence of the nature and severity of stress experienced and of the condition of fish at landing.

This thesis describes a series of discrete studies relating to the exercise physiology of juvenile snapper, *Pagrus auratus*, an iconic New Zealand finfish that comprises important recreational and commercial fisheries. In particular, we sought to characterise the capacity of snapper for sustained swimming activity, including how performance may differ between fish of different size or with environmental temperature; to determine the consequences of exhaustive exercise for both subsequent swimming activity, an important determinant of survival in escaping or discarded catch, and for tissue biochemistry, which ultimately determines product quality in harvested fish; to validate the use of laboratory-based simulations for the study of capture-related stress by comparing the response of laboratory-exercised snapper with commercially caught fish; and to determine the tolerance of snapper

to environmental hypoxia, and further, the possible consequences of hypoxia for swimming capacity and for recovery in fish retained for subsequent rested-type harvest.

The capacity of snapper for sustained swimming activity was characterised through the use of incremental exercise tests to determine critical swimming speeds, U_{crit}. Juvenile snapper (94-107 mm length, 16-157 g mass) demonstrated a strong swimming capacity, with individual fish attaining critical swimming speeds of up to 7.1 body lengths per second (bl s^{-1}). Swimming performance demonstrated an allometric association, with absolute critical speeds increasing with fish size, whilst relative performance favoured smaller fish. The relation was described by the function U_{crit} (m s^{-1}) = 0.003412 [length (mm)] + 0.2669. Critical swimming performance also exhibited variation in response to environmental variables. Thermal performance curves were evident in snapper acclimated to 12, 18 and 24 ^{o}C, with the suggestion of optimal performance at acclimation temperatures between 18 and 24 ^{o}C. Critical swimming performance was also significantly reduced during exposure to ambient oxygen tensions below 80 mmHg; at 40 mmHg, snapper attained only 21% of the critical swimming speeds observed under normoxic (150 mmHg) conditions.

In juvenile snapper (~75 g), exhaustive exercise resulted in severe metabolic, acid-base, haematological and hormonal perturbations, the nature of which were similar to those classically demonstrated in other strong-swimming fish species, especially salmonids. These included the depletion of glycogen from within the white muscle (WM) and the concomitant production of lactate, with a resultant lactacidosis of the plasma; recruitment of erythrocytes from the spleen; and the release of cortisol to the plasma. The recovery of these disturbances required 6 hours under laboratory conditions. As the stresses experienced by fish during commercial capture are often considered to be greater than those which can be induced during laboratory-based simulations, it was necessary to investigate whether the magnitude of the perturbations observed in laboratory-exercised snapper were an appropriate model of those of trawl-caught fish. In trawl-caught snapper (1100 g, 38 cm) obtained under commercially-relevant conditions (tow speed ~3.0 knots; duration 2.25-2.75 hours), the magnitude of the perturbations were greater than for laboratory-exercised fish. While the recovery of some metabolites was evident within the first 18 hours post-capture, their recovery was prolonged relative to laboratory-exercised fish; other metabolites, namely muscle glycogen and plasma cortisol, exhibited no signs of recovery. These observations suggest that the response of snapper to exhaustive exercise within the laboratory may

underestimate the severity of the response induced by commercial harvest. This is further suggested by post-capture mortality rates of 14%, whereas no mortality was observed following fatigue at U_{crit}.

Exhaustive exercise also resulted in the impairment of subsequent critical swimming performance. Immediately following fatigue, snapper (85-160 g) were capable of sustained swimming activity at speeds of up to 60-70% U_{crit}; however, critical swimming performance was reduced 30%, presumably due to limitations in WM function. There was no suggestion of the recovery of WM function within the first 30 minutes post-fatigue; thereafter, U_{crit} was progressively restored, such that snapper were able to repeat their initial swimming performance in a second U_{crit} test performed 2 hours after the conclusion of the first.

Snapper were moderately tolerant of hypoxia, oxygen-regulating at reduced oxygen tensions (<100 mmHg) by virtue of increased ventilatory rate and stroke volume, with a distinct bradycardia developing at PO_2 below 60 mmHg. Larger snapper appeared to possess a greater hypoxia tolerance than did smaller fish, with P_{crit} resolved to 77 in 20 g fish, and 50 mmHg in 150 and 230 g fish. Exposure to moderate hypoxia (60-80 mmHg) during recovery from an exhaustive exercise event constrained $MO_{2\ max}$ to 78% of that of normoxic fish, however did not appear to impede the return of MO_2 to routine levels.

The present study is the first to examine in detail the swimming performance of snapper, and the consequences of exhaustive exercise for physiological condition. By understanding the swimming capacities of snapper, it may be possible to refine harvesting practices (i.e. tow speeds) or utilise technologies (i.e. net design) such that the water velocities through the trawl net are within the range at which the fish can swim sustainably, minimising the extent of stress and fatigue experienced by fish, and hence their effects on both quality and survival. The study also demonstrates that whilst snapper experience significant physiological disturbance during commercial harvesting, including significant mortality, some fish demonstrate the potential for metabolic recovery, which may permit their retention in an on-board tank facility for subsequent rested-type harvest. Finally, the present work highlights a number gaps in our understanding of the link between harvesting conditions and fish condition, and makes a number of suggestions for future studies or directions.

CHAPTER 1
General introduction

1.1 An introduction to New Zealand's fishing industries

Fisheries are among the most vital of the world's industries – with an annual commercial catch in excess of 90 million tonnes, they account for 19% of the world's consumption of animal protein, and with an annual revenue of US$70 billion, provide direct employment for 200 million people worldwide (Botsford et al., 1997; Zabel et al., 2003; FAO, 2009). However, increasing demand for fish and fisheries products, in combination with socioeconomic and political pressures for development and expansion, is placing considerable pressure on fish stocks, and they are becoming increasingly vulnerable to unsustainable fishing practices (Zabel et al., 2003; Worm et al., 2009). The Food and Agriculture Organisation of the United Nations (FAO) considers 50% of the world's marine fisheries to be fully exploited, and a further 30% as being either overexploited, depleted, or recovering from depletion; only 20% are considered as being either under- or moderately exploited (FAO, 2009). Further, the FAO estimates that as much as 25% of the catch from wild fisheries is lost post-harvest, either through the discard of bycatch at sea (estimated at 7 million tonnes in addition to the landed catch) or the loss of product due to spoilage (Huss, 1995; Kelleher, 2005).

New Zealand fisheries are no exception. With an Exclusive Economic Zone (EEZ) of 4.4 million km^2 – the fourth largest in the world – commercial ventures total an annual catch exceeding 600,000 tonnes, and provide employment of more than 8000 full time equivalents (Ministry of Fisheries, 2008, 2011). Fisheries consistently represent New Zealand's 4^{th} or 5^{th} largest export earner; more than 90% of the commercial catch is exported, with a value of $1.26 billion for the 2010/2011 season (Ministry of Fisheries, 2011). Expansion within the fisheries industry therefore has the potential to be of significant benefit to the New Zealand

economy. However, many of New Zealand's major fisheries are already either overexploited or being maximally harvested; despite the introduction of the Quota Management System (QMS) in 1986, 18% of the stocks of known status, including stocks of snapper (*Pagrus auratus*), kahawai (*Arripis trutta*), bluenose (*Hyperoglyphe antarctica*) and orange roughy (*Hoplostethus atlanticus*), are considered to be overfished or depleted and requiring active rebuilding (Ministry for Primary Industries, 2013*c*). It is therefore not possible to stimulate industry growth simply through an increase in landings. Rather, adding value to the existing catch through improvements in product quality has the potential to significantly benefit both the profitability and sustainability of capture fisheries (Huss, 1995). Specifically, improvements in harvesting techniques could yield a higher quality product capable of commanding a higher price, reduce waste during processing, extend shelf-life, and allow the production of alternative seafood products for which the catch was previously deemed unfit for purpose (Forgan, 2009) – all of which offer clear financial benefits. Improved utilisation of the catch may also provide the opportunity to relieve pressure on vulnerable fish stocks, by reducing catch requirements whilst sustaining financial margins. In addition, improvements in the sustainability of harvesting techniques, in particular, minimising the capture and/or associated mortality of juvenile and non-target species caught and subsequently discarded as bycatch, may lead to higher sustainable yields through increased recruitment to adult populations (Diamond and Campbell, 2009).

There is a growing body of evidence that the peri-mortem physiology of an animal is directly related to the condition of its tissues post-mortem, and that the transition from viable tissue to food product is a function of the harvesting conditions (Oehlenschlager and Rehbein, 2009). In particular, harvesting techniques that result in stress, fatigue or physical injury are known to have adverse effects on the taste, texture, odour, appearance and shelf-life of the product (Wells, 1987; Lowe et al., 1993; Huss, 1995; Berg et al., 1997; Erikson et al., 1997; Sigholt et al. 1997; Jerrett et al., 1998; Thomas et al., 1999; Robb et al., 2000; Skjervold et al., 2001; Black et al., 2004; Roth et al., 2006; Bosworth et al., 2007; Tuckey et al., 2010). An understanding of the physiology of a species and the nature of any changes induced in response to harvest is therefore essential to the refinement of harvesting practices (Forgan, 2009). For trawl-based capture fisheries, knowledge of the exercise physiology and swimming capacities of the target species, which play key roles in determining the interaction of fish with fishing gears and thus the types and magnitudes of stressors experienced during

capture (He, 1993; Wardle, 1993; Breen et al., 2004; Winger et al., 1999, 2010), may provide an opportunity to make directed improvements in the capture process, such that fish may be landed in the best possible condition, providing the best platform on which improvements in post-harvest storage technologies can be realised. Furthermore, such knowledge may be important in ensuring the efficacy of bycatch-reduction devices, and in estimating the fate of fish that escape capture or are discarded as bycatch (Farrell et al, 2000; Davis, 2002; Breen et al., 2004; Suuronen et al., 2005; Broadhurst et al., 2006).

1.2 Cellular metabolism and energy production

All cells must expend energy to perform vital cellular activities and maintain homeostasis. Adenosine triphosphate (ATP) is the "energy currency" of the cell (Garrett and Grisham, 2005); hydrolysis of its terminal phosphate group releases free energy that can be coupled to numerous exergonic reactions. The continued provision of ATP is facilitated by the phosphorylation of adenosine diphosphate (ADP) during one or more of the following sets of reactions – the hydrolysis of high-energy phosphagens, aerobic catabolism and anaerobic catabolism (Hochachka, 1985; Garrett and Grisham, 2005; Champe et al., 2008).

1.2.1 Hydrolysis of high-energy phosphagens

High energy phosphates provide the most rapid mechanism for the production of ATP (Hochachka, 1985). The primary substrate in these reactions is phosphocreatine (PCr); hydrolysis of PCr by creatine phosphokinase (CPK) and the subsequent donation of the phosphate group to ADP replenishes ATP (Fig. 1.1). High CPK activity in tissues such as glycolytic skeletal muscle (see Section 1.3.3) facilitates the regeneration of ATP at rates sufficient to meet demand during activities such as burst exercise, where turnover may increase 100-fold over resting levels (Hochachka and McClelland, 1997). However, as PCr is stored in only limited quantities within the cell (~30 μmol g^{-1}in skeletal muscle; Hochachka, 1985), it is rapidly depleted, and the subsequent provision of ATP must occur through other metabolic pathways.

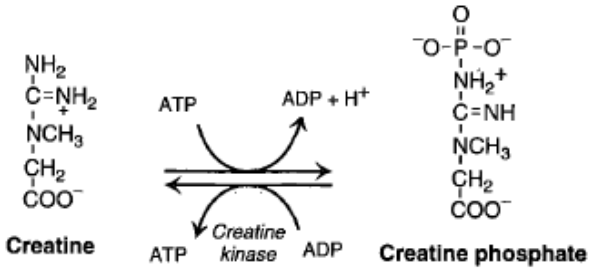


Figure 1.1. Phosphorylation of ADP by creatine phosphate to replenish ATP. Modified from Champe et al. (2008).

1.2.2 Aerobic catabolism

Aerobic catabolism involves the production of ATP through the oxidation of carbohydrate, lipid and protein substrates. These reactions, which are dominant when the oxygen supply is sufficient, are described in detail by Garrett and Grisham (2005), and are summarised in Figure 1.2.

Aerobic catabolism comprises two universal stages, regardless of substrate: the Krebs (or tricarboxylic acid) cycle and oxidative phosphorylation. Substrates typically enter the Krebs cycle as acetyl coA. Carbohydrates, especially glycogen, are first hydrolysed to glucose, after which they are converted via glycolysis to pyruvate, before oxidation to acetyl CoA. Lipids (as triglycerides) are degraded to glycerol and fatty acids by various lipases; glycerol is subsequently converted to the glycolytic intermediate, glyceraldehyde-3-phosphate, while β-oxidation of the fatty acid chain sequentially removes two-carbon fragments as acetyl coA. Proteins must first be digested into their constituent amino acids; the catabolic pathways of different amino acids vary, and following deamination, derivatives may enter the Krebs cycle as cycle intermediates, pyruvate, or acetyl coA itself.

The Krebs cycle functions as a "metabolic furnace" (Campbell and Reece, 2005), oxidising acetyl coA to CO_2 and H_2O. Although each cycle yields only one molecule of ATP, it is the reduction of the coenzymes nicotinamide adenine dinucleotide (NAD^+) and flavin adenine dinucleotide (FADH) to NADH and $FADH_2$ respectively that allows the realisation

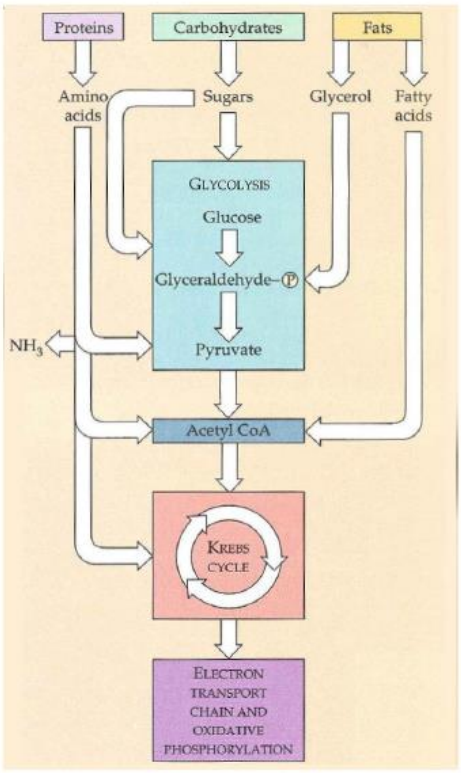
of the energy content of the substrate molecule. Electrons liberated by the Krebs cycle are transferred via NADH and $FADH_2$ to the electron transport chain (ETC), a group of four protein complexes embedded in the inner mitochondrial membrane. These complexes, and their associated proteins, serve as electron carriers, alternating between their oxidised and reduced states as they accept and donate electrons between neighbouring complexes. The chain culminates in the reduction of molecular oxygen; it is this role as the terminal electron acceptor that makes oxygen vital to aerobic metabolism. The passage of electrons along the ETC and their excretion with the resultant H_2O allows for the regeneration of NAD^+ and FADH, with two fundamental consequences: firstly, oxidation of these two coenzymes is essential for the continuing function of the Krebs cycle, and secondly, the free energy released by their oxidation is coupled by the ETC complexes to the pumping of protons into the mitochondrial intermembrane space. The resulting proton gradient is used to drive ATP synthesis. The complex responsible for ATP synthesis, ATP synthase, is the only site in the membrane freely permeable to H^+; the resultant movement of protons through a narrow channel in the structure of ATP synthase drives a pattern of conformation changes in the complex subunits, resulting in the phosphorylation of ADP to create ATP. The processes of electron transport and ATP synthesis are together referred to as oxidative phosphorylation.

Oxidative phosphorylation is the most efficient means of generating ATP; complete oxidation allows the realisation of a substrate's energetic content, although the exact yield of ATP depends on the substrate. The complete oxidation of one molecule of glucose may yield a net production of 36 molecules of ATP – 2 produced during glycolysis and 34 from oxidative phosphorylation by ATP synthase.

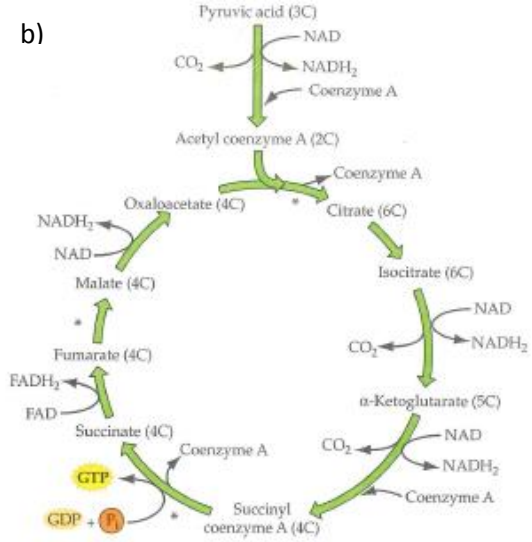
1.2.3 Anaerobic catabolism

If the oxygen supply becomes limited, the ETC and oxidative phosphorylation are compromised; without oxygen to act as the terminal electron acceptor, electrons accumulate and ETC constituents become fully reduced (Garrett and Grisham, 2005). Without the release of free energy associated with the oxidation of coenzymes, a proton gradient cannot be established, and ATP synthesis ceases. Under these conditions, carbohydrates represent the only available substrate for energy production; the oxidation of glucose to pyruvate may

a)



b)



c)

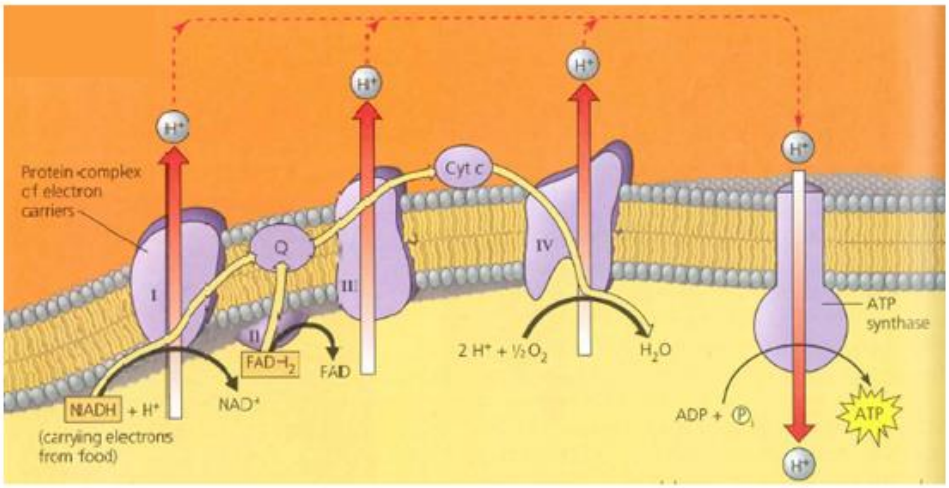


Figure. 1.2. Overview of the production of ATP under aerobic conditions. a) Schematic representation of the pathways by which carbohydrate, lipid and protein substrates enter the Krebs cycle during aerobic catabolism. b) During ATP production by oxidative phosphorylation, substrates enter the Krebs cycle as pyruvate, acetyl coA, or cycle intermediates. The oxidation of intermediates yields the reduced coenzymes NADH and $FADH_2$, which subsequently reduce the enzyme complexes of the electron transport chain. c) The passage of electrons through the ETC culminates in the reduction of molecular oxygen. Free energy released by the oxidation of NADH and $FADH_2$ is coupled to the generation of a proton gradient, which is subsequently used to drive ATP synthesis by ATP synthase. Figures modified from Hill et al. (2004) and Campbell and Reece (2005).

proceed anaerobically, and yield 2 ATP for each molecule of glucose metabolised. These reactions still require the regeneration of NAD^+; during anaerobic catabolism, pyruvate acts as the terminal electron acceptor, and is reduced to lactic acid, allowing the maintenance of redox balance and continuation of glycolytic reactions (Hill et al., 2004; Fig. 1.3). As lactic acid can be metabolised only in the presence of oxygen, it accumulates within the cell, dissociating into lactate ions and protons.

Anaerobic glycolysis may also be recruited during periods in which the aerobic capacity of the cell is exceeded; if the rate of ATP provision by oxidative phosphorylation is insufficient to meet demand, production is supplemented by PCr hydrolysis and anaerobic glycolysis. In the case of either insufficient aerobic capacity or oxygen limitation, the dependence on carbohydrates and the low yield of ATP per unit substrate renders carbohydrate reserves vulnerable to depletion. Further, the accumulation of protons associated with the prolonged utilisation of anaerobic metabolism alters intracellular pH, with consequences for cellular function, in particular, the activities of various enzymes.

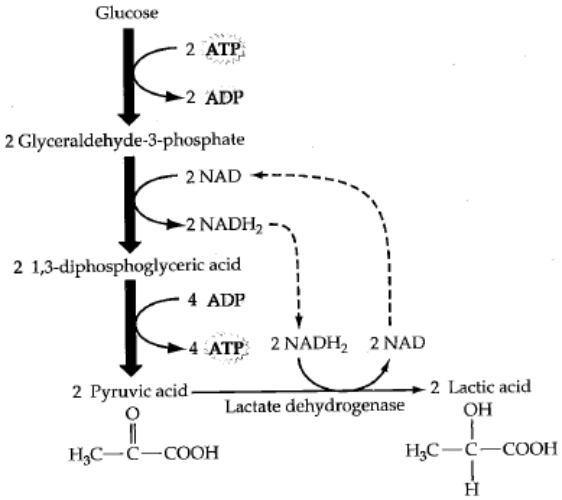


Figure 1.3. The reactions of glycolysis under anaerobic conditions. The reduction of pyruvic acid to lactic acid by lactate dehydrogenase (LDH) allows the regeneration of NAD^+ and maintenance of redox balance. From Hill et al. (2004).

1.3 Teleost skeletal muscle – structure and function

Muscle is a specialised tissue capable of transmitting force through a series of coordinated contractions. Muscle tissues are classified into three types – skeletal, cardiac and smooth – with each offering different physical and functional characteristics (Sherwood et al., 2005). In teleost fish, the skeletal muscle comprises as much as 60% of the total body mass, a requirement of supporting locomotion in a dense medium (Bone, 1978).

1.3.1 Muscle structure

Skeletal muscle consists of numerous, multinucleate, cellular units called fibres, lying in parallel and arranged into blocks or myotomes (Bone, 1978; Videler, 1993; Sherwood et al., 2005). Neighbouring myotomes are held together by collagenous myosepta. The ability of muscle fibres to develop tension and shorten is derived from contractile elements called myofibrils; myofibrils are themselves composed of several types of myofilament – thick and thin filaments – arranged into repeating units known as sarcomeres (Fig. 1.4). Thick filaments consist primarily of the protein myosin; identical subunits are arranged with "tails domains" intertwined and "head domains" projecting at one end in a regular, staggered arrangement. Interleaved with the thick filaments are thin filaments, composed of three proteins: actin, tropomyosin and troponin. Globular actin subunits interact to form strands of fibrous actin. The double-helical tropomyosin wraps around the actin filament, and in conjunction with troponin, acts to regulate the interaction between the key sites involved in contraction – the head domains of the myosin units and the myosin-binding sites of actin.

1.3.2 Muscle contraction

Muscle contraction is initiated by the process known as excitation-contraction coupling (EEC) (Sherwood et al., 2005). Reception of a neural input at the neuromuscular junction results in the release of acetylcholine (Ach) from the nerve terminal; reception of Ach at the myocyte results in the depolarisation of the sarcolemma. Propagation of the action potential across the surface of the fibre and through a network of membrane invaginations known as the transverse (or T) tubules, causes the release of calcium ions from the lateral sacs of the

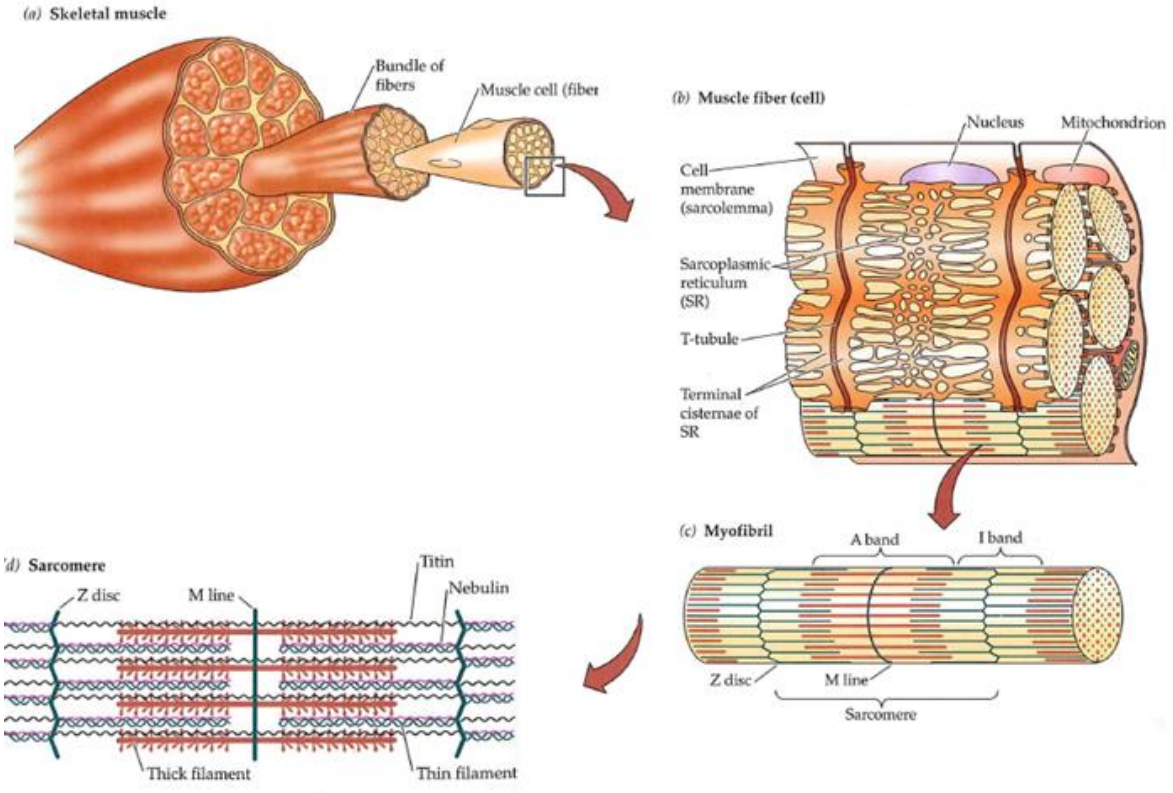


Figure 1.4. The structural organisation of skeletal muscle. Modified from Hill et al. (2004).

sarcoplasmic reticulum (SR). This mass release of Ca^{2+} is essential for contraction to occur; the binding of Ca^{2+} to troponin causes a conformational change that results in the displacement of tropomyosin from the myosin-binding sites of the actin filament. Subsequent interaction of actin and myosin permits contraction according to the "sliding filament model": the formation of the reversible "crossbridge" interaction between actin and myosin causes a change in the angle at which the myosin heads project, pulling the actin filaments towards the centre of the sarcomere, effectively shortening the functional muscle unit. Dissociation of the actin-myosin interaction is facilitated by Mg^{2+}-dependent myosin ATPase, and in the continued presence of ATP and Ca^{2+}, the myosin head will detach, then reattach to a distal actin molecule. This cross-bridge cycling allows full contraction to occur.

As electrical activity in the innervating motor neuron ceases, so too does the release of Ach; residual Ach at the junction is degraded by acetylcholinesterase (AchE). The repolarisation of the sarcolemma arrests the release of further Ca^{2+} from the lateral sacs, and free Ca^{2+} within the sarcoplasm is rapidly returned to the SR by the activity of the SR Ca^{2+}ATPase. In the absence of sarcoplasmic Ca^{2+}, the troponin-tropomyosin complex resumes its inhibitory position; the subsequent lack of actin-myosin interactions and the elastic properties of the structural protein titin allow the thick and thin filaments to slide back past each other, as the muscle relaxes to its resting conformation.

1.3.3 Muscle fibre types

Fish skeletal muscle, like that of other vertebrates, can be divided into two distinct fibre types – oxidative and glycolytic - more commonly designated red and white fibres, on account of their appearance. In fish, however, the two fibres types are highly compartmentalised. The cone-shaped myotomes of the white muscle (WM), which account for 80-95% of the total muscle mass, are stacked to form a W pattern, within which the muscle fibres form complex helical trajectories (Fig. 1.5; Goldspink, 1977; Bone, 1978; Altringham and Ellerby, 1999). This arrangement permits all of the fibres to contract at the same rate and extent as the body flexes, regardless of their position relative to the vertebral column; as force production is a function of both contraction velocity and sarcomere excursion, all fibres are therefore capable of generating maximum force (Bone, 1978; Videler, 1993). The majority of the remainder of the muscle is present as red muscle (RM), which forms a thin wedge of superficial tissue running the length of the fish, near to the lateral line. The uniform distance between these fibres and the vertebral column means these red fibres lie in parallel to the long axis of the fish (Bone, 1978; Altringham and Ellerby, 1999). In many species, a third fibre type – the so-called "pink muscle" – is also present, and is located around the periphery of the red muscle (Johnston et al., 1975; Davison et al., 1976; Goldspink, 1977).

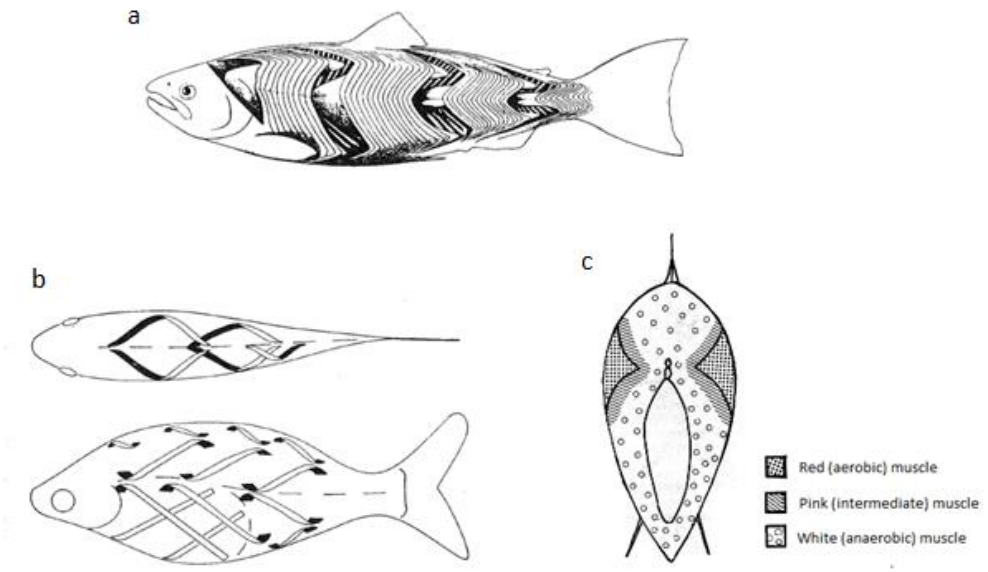


Figure 1.5. Schematic illustration of the arrangement of the musculature within a typical teleost. a) Illustration of the W-shaped myotomes of the white muscle. b) Dorsal and lateral views showing the helical trajectories of the WM fibres. c) Cross-section through the trunk, illustrating the segregation of red, white and pink muscle fibre types. Modified from Videler (1993), Bone (1978) and Johnston et al. (1975).

Red muscle fibres are relatively slow to contract, and are used to support low-intensity activity, which may be sustained for extended periods; their metabolism is therefore predominantly aerobic, fuelled by lipid and some carbohydrate (Pritchard et al., 1971; Johnston and Goldspink, 1973; Richards et al., 2002*b*). The structure of the RM confers a high aerobic capacity, with the highly vascularised fibres being small in diameter (providing a high surface area to volume ratio), and containing high concentrations of myoglobin, lipid and glycogen. Mitochondrial density is high (25-35% of fibre volume; Sänger, 1992) and the mitochondria themselves are larger and contain higher densities of cristae than those of WM, maximising the concentration of oxidative enzymes (Johnston et al., 1975, 1977; Patterson et al., 1975; Davison and Goldspink, 1977; Bone, 1978; Sänger, 1992). In contrast, WM fibres are larger in diameter than RM fibres, and are characterised by high myofibrillar densities and a myofibrillar ATPase activity approximately three times to four times that of the RM (Johnston et al., 1975, 1977; Davison et al., 1976; Bone, 1978; Sänger, 1992); the resultant increase in the speed and strength of contraction is used to support high intensity, burst

activity. While the basal metabolism of WM tissue is aerobic, the rapid demand for ATP during burst activity readily exceeds the low aerobic capacity of the fibres, and is instead met by the anaerobic metabolism of intracellular reserves of PCr and glycogen (Johnston and Goldspink, 1973; Milligan, 1996; Hochachka and McClelland, 1997; Kieffer, 2000). Given its functional dependence on anaerobic rather than aerobic generation of ATP, WM is poorly vascularised and contains few mitochondria and little myoglobin, instead possessing high levels of glycolytic enzymes (Johnston et al., 1975, 1977; Bone, 1978). White muscle is therefore highly susceptible to fatigue resulting from substrate depletion and/or the accumulation of metabolic byproducts. Pink muscle fibres show biochemistry and function intermediate to red and white fibres: of moderate size, myofibrillar density and vascularisation, and containing intermediate levels of both oxidative and glycolytic enzymes, they can utilise both aerobic and anaerobic metabolism in accordance with the intensity of activity. The little work carried out on this fibre type suggests its use is species-specific (Patterson et al., 1975; Davison et al., 1976; Johnston et al., 1977).

1.4 Locomotion and exercise metabolism

1.4.1 Locomotion in fish

To move through a medium as dense as water, aquatic organisms must generate thrust (Rome et al., 1993; Bone et al., 1995). Different species of fish display a range of strategies for locomotion, from undulatory movements in which the whole body participates, to movements powered solely by the pectoral, dorsal and/or anal fins (Lindsey, 1978). The majority of fish, however, exhibit carangiform or subcarangiform swimming, where lateral undulations of the trunk and tail are powered by the sequential activation of myotomal muscle blocks, and the tension subsequently transmitted to the vertebral column and the skin via the inextensible myosepta. Inertial forces of the water resist the resulting movement of the caudal fin, generating an equal and opposite force on the fish, propelling it forwards (Rome et al., 1993; Videler, 1993; Bone et al., 1995; Altringham and Ellerby, 1999).

The power output of the muscle required to overcome drag during locomotion is proportional to (swimming velocity)$^{2.8}$ (Bone et al., 1995). Sarcomere excursion is

independent of swimming velocity, while tail-beat frequency increases linearly with speed; hence the velocity with which the muscle contracts must also increase with swimming velocity (Bainbridge, 1958; Rome et al., 1990, 1992). In addition to the increasing power requirements associated with overcoming drag at increasing swimming speeds, the force generated by muscle fibres is inversely related to the rate at which they contract (Rome et al., 1984, 1990; Videler, 1993), and so additional fibres are increasingly recruited at higher swimming speeds. The general pattern of fibre recruitment appears universal – oxidative fibres are recruited at lower swimming speeds, followed by the powerful glycolytic fibres, although the specific velocities of recruitment are species- and temperature-specific (Hudson, 1973; Johnston et al., 1977; Bone et al., 1978; Rome et al., 1984, 1985, 1992; Sisson and Sidell, 1987; Altringham and Ellerby, 1999).

The increasing workload of the lateral musculature with increasing swimming velocities elevates oxygen consumption, such that $\dot{M}O_2$ increases exponentially with swimming speed (Brett, 1964, 1965; Videler, 1993; Lee et al., 2003*a*, 2003*b*; Claireaux et al., 2006), increasing as much as 12-15 fold over resting rates during strenuous and sustained activity (Brett, 1964). The increased provision of oxygen to musculature is facilitated by adjustments in cardiovascular and respiratory function (Heath, 1973; Taylor et al., 1996). The specific mechanisms by which the cardiorespiratory system augments oxygen delivery may include elevations in heart rate and stroke volume and ventilatory stroke rate and volume (Heath, 1973; Stevens and Randall, 1967; Randall, 1982; Kolok and Farrell, 1994; Steinhausen et al., 2008). In addition, an increase in blood pressure facilitates lamellar recruitment to further increase the capacity for oxygen extraction (Booth, 1978; Randall, 1982), and changes in systemic blood flow preferentially direct oxygenated blood toward the working musculature (Randall and Daxboeck, 1982).

1.4.2 Sustained, prolonged and burst swimming

Fish swimming may be classified into three main categories – sustained, prolonged and burst – reflective of the intensity of the exercise, with associated constraints relating to the time that activity can be maintained, the biochemical fuels used and consequences of the activity (reviewed by Beamish, 1978; Hammer, 1995; Kieffer, 2000). Sustained swimming is defined as that which a fish can maintain for extended periods (>200 minutes) and which

does not result in muscular fatigue. These slow swimming speeds are powered by the red muscle using ATP derived from the oxidation of substrates, typically lipids. Sustained swimming causes little physiological disturbance to the muscle tissue (Richards et al., 2002*b*), since oxidation end products (CO_2 and H_2O) are readily excretable, and the efficiency of oxidative phosphorylation maximises the energetic return from consumed fuel reserves.

The highest speeds a fish can attain are termed burst speeds, and can be maintained for only short periods (<20 seconds). Burst activity is facilitated by the rapid rate and high force of contraction of the white muscle. The abrupt increase in demand for ATP within the WM results in a change in the relative concentrations of ATP and ADP, stimulating the hydrolysis of PCr by CPK. PCr reserves are rapidly depleted (up to 90%; Dobson and Hochachka, 1987; Milligan 1996), after which ATP is generated through anaerobic glycolysis. The dependency of burst activity on anaerobic metabolism results in the depletion of glycogen (also up to 90%; Milligan, 1996), and a concomitant lactacidosis of the muscle and blood, which in turn incites disturbance in acid-base, ionic and osmotic balance (Wood, 1991). Ultimately, burst activity results in fatigue, characterised by the reduced capacity of the muscle for mechanical work. While the etiology of fatigue is complex, the accumulation of metabolites – in particular, H^+ and P_i – is thought to play a key role in the impairment of muscle function, for example, through compromised excitation-contraction coupling and/or development of force by actomyosin (Altringham and Johnston, 1985; Mutungi and Johnston, 1988; Sjøgaard, 1990; Westerblad et al., 2002; Green, 2004; Vandenboom, 2004).

Prolonged swimming describes the range of swimming velocities intermediate to sustained and burst swimming. This type of activity is powered by both aerobic and anaerobic metabolism, with the relative contributions of each depending on the given swimming speed, and determining the time for which activity can be sustained before ending in fatigue. The functional limits that demarcate the transition from sustained to prolonged activity remain unresolved (Claireaux et al., 2006; Taylor et al., 2008), but are likely defined by the capacity of the cardiorespiratory system to supply sufficient oxygen to the muscle, or even to the heart itself, given its dependence on venous oxygen content (Farrell, 2002, 2007*a*), or by limitations in the performance of the RM, in particular its ability to generate sufficient force and hence the need to recruit WM in order to further increase swimming velocity (Rome et al., 1990; 1993; Sidell and Moerland, 1989; Randall and Brauner, 1991).

1.5 The role of exercise physiology in capture processes

The majority of capture methods used in the commercial harvest of wild fish result in the stress, injury and/or fatigue of the fish, with the severity of the stress depending on the method of capture (Farrell et al., 2000; Black, 2002; Davis, 2002). In trawl-based fisheries, the stressors experienced by fish during capture may include exhaustive exercise, confinement, crowding, impact and crush injuries, barotrauma, scale loss and hypoxia and/or asphyxia (Davis, 2002; Ryer, 2004; Suuronen, 2005; Broadhurst et al., 2006). The interaction of fish with the fishing gears, and hence the particular stressors experienced, can be complex, varying in relation to a myriad of intrinsic and extrinsic factors, including the species, size and physical condition of the fish, water temperature, ambient light intensity, sea conditions, tow speed and duration, and net configuration. The swimming abilities of fish – including maximum speed, endurance performance and manoeuvrability – also play a key role determining the interaction between fish and fishing gears, with implications for both the efficiency and selectivity of fishing gears (He, 1993; Wardle, 1993; Winger et al., 1999, 2010; Breen et al., 2004).

The importance of endurance swimming in the capture process has been described by Wardle (1993) and Winger et al. (2010). As the fishing vessel approaches, the otter boards and sweeps of the net herd fish into the trawl path. Once inside the tow mouth, most fish demonstrate a classic optomotor response, orienting in the direction of the tow and attempting to keep pace with the approaching gears. The period of time a fish is able to maintain its position is dependent upon its maximum sustainable swimming speed, relative to the tow speed; if the tow speed is near or exceeds endurance capacity, the fish is forced to recruit its glycolytic musculature, and as it begins to fatigue, it moves back into the body of the net. Within the net, contact with the net mesh and/or the accumulating catch stimulates burst-type escape behaviours, which ultimately result in metabolic exhaustion, and the fish is swept back into the codend. Fish within the accumulating catch are often crushed by other fish, and ventilation is impaired as fish are pinned against the mesh by the water current or by other fish; consequently these fish are often dead or dying at landing.

1.5.1 Implications of capture for product quality

1.5.1.1 Post-mortem changes in fish muscle

Following the death of the fish, the tissue remains viable for a period; however the cessation of tissue perfusion means residual metabolic processes are fuelled anaerobically, and with no potential for recovery (Thomas et al., 1999; Tejada, 2009). Cellular reserves of PCr, ATP and glycogen are consumed until they are exhausted, or until concomitant changes in pH arrest enzyme activity. Ultimately, ATP production fails, and cells cease to function as homeostasis is lost (Dalgaard, 2002; Tejada, 2009). With the loss of cell viability, the tissue undergoes a series of physical and biochemical changes that directly impact product quality and shelf-life (Dalgaard, 2002; Oehlenschlager and Rehbein, 2009). The changes that occur within the WM have traditionally been separated into four stages: development of rigor, resolution of rigor, autolysis and bacterial spoilage (Sigholt et al., 1997). The rate and extent of these processes is influenced by a multitude of factors, including species, peri-mortem condition, method of slaughter, handling and processing techniques, and storage conditions, especially temperature (Oehlenschlager and Rehbein, 2009). In general, endogenous autolytic reactions are associated with the initial loss of freshness attributes, while bacterial processes are largely responsible for the development of overt spoilage, which ultimately limits shelf-life (Dalgaard, 2002).

The first physically observable process is the development of *rigor mortis*. Rigor develops as ATP reserves are depleted, resulting in the failure of the SR Ca^{2+}-ATPase to maintain calcium gradients; Ca^{2+} diffuses from the SR to the sarcoplasm, where it facilitates the formation of inextensible actomyosin, which in the absence of ATP, cannot be resolved (Huss, 1995; Berg et al., 1997; Oehlenschlager and Rehbein, 2009; Tejada, 2009). After a period of hours to days (depending on pre- and post-harvest conditions), rigor is resolved and the muscle begins to soften, although the desirable, pre-rigor elasticity of the fillet is lost (Huss, 1995; Oehlenschlager and Rehbein, 2009). Endogenous proteases, including calpains, cathepsins and collagenases, are activated by changes in the cellular environment, including reduced pH and elevated Ca^{2+}, and degrade structural proteins involved in both the myofibrillar structure, and interaction with cytoskeletal elements and connective tissues (Huss, 1995; Dalgaard, 2002; Oehlenschlager and Rehbein, 2009; Tejada, 2009).The

degradation of myofibrillar elements such as the Z line results in the softening of the tissue, while the disintegration of myosepta result in the tendency of the tissue to gape.

The resolution of rigor is approximately coincident with the onset of other autolytic processes, in particular, nucleotide degradation (Huss, 1995). Endogenous enzymes catalyse the sequential breakdown of ATP to ADP, adenosine-5'-monophosphate (AMP), inosine-5'-monophoshpate (IMP), inosine (Ino), hypoxanthine (Hx), xanthine (X) and finally, to uric acid (U). While the degradation of ATP to IMP occurs rapidly, the formation of subsequent derivatives is relatively slow; IMP therefore accumulates in the tissue. IMP is a known flavour enhancer, contributing to the pleasant "umami" taste of fresh fish (Huss, 1995; Tuckey et al., 2010; Tejada, 2009); its subsequent degradation is therefore associated with a loss of desirable flavours. Furthermore, concomitant production of Ino and Hx is associated with the development of bitter flavours (Huss, 1995). The K-value of a tissue represents the ratio of Ino and Hx to the total pool of ATP-related compounds (Satio, 1959), and is used extensively as an index of freshness, especially in Japanese commercial markets (Tejada, 2009). Acceptable K-values differ between species and the intended use of the product.

The highly unsaturated nature of fish lipids renders them susceptible to the oxidative and hydrolytic reactions implicated in the spoiling of fish flesh (Dalgaard, 2002; Oehlenschlager and Rehbein, 2009). Free radical species, such as superoxide produced by the mitochondria or iron released from haemoglobin or myoglobin, incite a series of self-propagating oxidation reactions, which yield short-chain carbonyl compounds, including aldehydes, ketones, and carboxylic acids; these compounds contribute to the rancid odours and flavours of fish as it begins to spoil (Tuckey, 2008; Oehlenschlager and Rehbein, 2009). Lipid radicals may also be formed enzymatically by endogenous lipoxygenase. Lipids are also susceptible to hydrolysis by lipases leached from the digestive tract, excreted by microbes or inherent within the muscle tissue itself; the resulting free fatty acids (FFAs) are particularly susceptible to oxidation and hence exacerbate the oxidative production of short chain spoilage compounds (Huss, 1995; Tuckey, 2008). In addition, FFAs may bind to muscle protein, contributing to the deterioration of muscle texture (Oehlenschlager and Rehbein, 2009).

The muscle of live, healthy fish is sterile; however, the collapse of the immune system post-mortem allows bacteria present in the skin, gills and gut to penetrate the fillet (Huss, 1995). Subsequent bacterial proliferation is associated with the overt spoilage of fish, as

certain strains facilitate the decarboxylation of amino acids to biogenic amines including histamine, cadaverine and putrescine, and the reduction of trimethylamine oxide (TMAO) to dimethylamine (DMA) and formaldehyde (FA). These compounds are responsible for the characteristic "fishy" and "off" odours of spoiled fish (Huss, 1995; Oehlenschlager and Rehbein, 2009). Bacterial processes are largely independent of the metabolic condition of the tissue, although poor texture quality may allow the more rapid penetration of bacteria and increase the availability of amino acid substrate; rather, storage conditions – especially temperature – play a significant role in determining the rate of bacteria-mediated spoilage reactions.

1.5.1.2 Implications of capture for product quality

There is a growing body of evidence demonstrating the importance of pre-harvest physiology in determining post-mortem tissue condition. As the onset of autolytic processes is associated with the loss of homeostasis that results from the depletion of cellular energy reserves, the rate at which post-mortem changes in tissue quality progress depends on the ability of the tissue to defend its energy reserves (Black et al. 2004). The preservation of endogenous ATP and PCr during the rested harvest of cultured fish extends the period for which residual metabolic processes are maintained, and thus delays the onset of tissue degradation (Jerrett et al., 1998; Black et al., 2004; Bosworth et al., 2007; Tuckey et al., 2010). In contrast, the stress and fatigue experienced by fish during capture depletes energy reserves; further, exhaustive exercise may induce severe acid-base, ionic and hormonal perturbations (Wood, 1991; Milligan, 1996), resulting in the prolonged elevation of energy expenditure as the cell attempts to reinstate homeostasis (Wells, 1987; Black, 2002), further accelerating the onset of autolytic processes.

The rapid depletion of ATP and PCr in the WM of fish that experience peri-mortem stress or fatigue (Lowe et al., 1993; Berg et al., 1997; Erikson et al. 1997; Sigholt et al. 1997; Tuckey et al., 2010) accelerates both the development and resolution of *rigor mortis* (Lowe et al., 1993; Berg et al., 1997; Erikson et al., 1997; Sigholt et al., 1997; Jerrett et al., 1998; Thomas et al. 1999; Robb et al. 2000; Skjervold et al., 2001; Roth et al., 2006). In addition, the muscle also develops a greater rigor tension (Berg et al., 1997; Erikson et al., 1997; Robb et al., 2000; Skjervold et al., 2001; Roth et al. 2006), applying increased strain to the

collagenous myosepta and increasing their susceptibility to disintegration on the resolution of rigor, resulting in the gaping of the fillet (Huss, 1995; Erikson, 1997; Robb et al., 2000; Roth et al., 2006). Nucleotide degradation is also accelerated, with increased K-values (Lowe et al. 1993; Erikson et al., 1997; Tuckey et al., 2010) indicating the rapid progression from fresh umami flavours towards less favourable taste profiles.

As a result of the recruitment of anaerobic glycolysis, the pH of WM in fish that experience stressful or exhaustive harvest is significantly reduced (Sigholt et al., 1997; Jerrett et al., 1998; Thomas et al., 1999; Robb et al., 2000; Kiessling et al., 2004; Tuckey et al., 2010). Reduction in pH alters the surface potential of myofibrillar proteins, resulting in their partial denaturation and reducing water holding capacity (Huss, 1995). The resultant increase in drip loss (Kiessling et al. 2004; Roth et al. 2006) may lead to a reduction in fillet mass and the production of unattractive exudates (Dalgaard, 2002). Changes in water content are also associated with changes in the refractory behaviour of light, altering the appearance of the fillet (Robb et al., 2000; Dalgaard, 2002), and in some species, is associated with the development of a tough texture (Huss, 1995). Low pH has also been implicated in the promotion of lipid and myoglobin oxidation (Dalgaard, 2002).

While it is evident that changes in peri-mortem condition are associated with changes in post-mortem quality, the majority of these changes are evident during only the first few days of storage; although degradation proceeds more slowly in fish where cellular energy reserves are preserved through low-stress harvest, the changes ultimately proceed to a similar point (Fletcher et al., 2003). Further, the preservation of energy reserves does not offer protection against bacterial proliferation and spoilage which is ultimately responsible for limiting shelf life (Fletcher et al., 2003; Bosworth et al., 2007; Tuckey et al., 2010). Thus, while improvements in harvesting technique have the potential to maximise the freshness and quality attributes during the first few days post-harvest, extension of shelf-life requires the manipulation of storage conditions and development of post-harvest technologies.

1.5.2 Selectivity of trawl gears

High rates of incidental capture of juvenile and/or non-target species, together known as bycatch, is one of the most significant issues facing commercial fisheries (Olla et al., 1997;

Davis, 2002). Following an extensive review of the literature and industry documentation, Alverson et al. (1994) estimated that the global rate of discard of juvenile and non-target fish amounted to some 27 million tonnes. More recently, Kelleher (2005) estimated the discard of bycatch to be 7 million tonnes, or 8% of the total global catch. Kelleher (2005) reasoned that while some of the difference between the estimates could be attributed to methodological and analytical differences, it also reflected a real reduction in the discard rate during the period between the two studies, through a combination of the increased use of bycatch reduction devices (BRDs), the introduction and enforcement of discard regulations, changes in fishing effort within some fisheries, and increased retention of previously discarded catch, as a result of improvements in processing technologies and expanding markets. Fishing methods that utilise towed gears, especially trawl fisheries, are associated with the capture of disproportionately high volumes of bycatch; for example, shrimp and demersal trawl fisheries account for more than 50% of global bycatch rates, despite representing only 22% of total landings (Kelleher, 2005).

In trawl-based fisheries, efforts to reduce the taking of bycatch have focused on the use of increased minimum mesh size, alternative mesh geometry (i.e. square mesh) within the codend, and/or the inclusion of sorting grids, to facilitate the escape of undersized fish at depth (Chopin and Ariomoto, 1995; Davis, 2002; Ryer, 2004; Ryer et al., 2004; Suuronen, 2005). However, as these measures do not preclude or deter non-target or juvenile fish from entering the tow, fish remain exposed to the above-mentioned capture-related stresses (Chopin and Ariomoto, 1995). Further, as the negotiation of selection devices requires deliberate, often high-intensity swimming, fish that do not engage these devices until after having experienced significant stress, exercise and/or injury may have a reduced capacity for escape (Breen et al., 2004; Suuronen, 2005; Winger et al., 2010). To this end, an understanding of the swimming capacity of juvenile fish may be of value when evaluating the design and potential efficiency of bycatch-reduction devices.

1.5.3 Escapee and discard mortality

Efforts to improve the selectivity of trawl gears are only of value if those fish that do escape survive the encounter (Chopin and Arimoto, 1995). Mortality of fish escaping from trawl gears can be extensive, as the direct result of severe metabolic disturbance associated

with exhaustive exercise, severe stress and/or physical injury sustained within the trawl or during escape (Wood et al., 1983; Suuronen et al., 1995, 1996*a*, 1996*b*; Sangster et al., 1996; Ryer, 2004; Suuronen, 2005; Olsen et al., 2012). Estimates of mortality in escapee fish are hugely variable – between 0 and 100% – reflective of differences in experimental and capture methodologies, environmental conditions, and inherent species-specific differences in susceptibility to mortality (see Chopin and Arimoto (1995) and Broadhurst et al. (2006) for reviews). Further, mortality may be delayed, potentially occurring over an extended period following escape or release: capture mortality was observed up to 6 days post-capture in mackerel (*Scomber scombrus*; Lockwood et al., 1983), 14 days in walleye pollock (*Theragra chalcogramma*; Olla et al., 1997) and Baltic herring (*Clupea haengus*; Suuronen et al., 1996*a*,1996*b*) and 30 days in Pacific halibut (*Hippoglossus stenolepis*; Davis and Olla, 2001). Delayed mortality may therefore represent a significant source of unobserved mortality. Mortality may also be size dependent, with smaller fish often showing greater sensitivity to capture-related stressors (Suuronen et al., 1996*a*, 1996*b*; Suuronen, 2005; Stewart, 2008). Further, fish retained within the codend and landed prior to subsequent discard may experience additional stress associated with crush injury, air exposure and/or barotrauma (Davis, 2002; Suuronen, 2005). For example, the survival of juvenile snapper caught during a trawl survey was inversely related to the duration of air exposure following landing, with mortality rates increasing from <30% after an exposure period of 5 minutes, to 85% after 15 minutes and 100% after 30 minutes (Sumpton and Jackson, 2005). Mortality was similarly correlated with capture depth (Stewart, 2008), with no mortality in snapper caught at depths of <21 m, increasing to 39% at 30-44 m and 55% at 45-59 m.

Discard and escapee fish that experience sub-lethal metabolic perturbations and/or physical injury during capture or escape may remain vulnerable to indirect mortality as a result of stress-induced physiological and behavioural deficits. Impaired swimming performance, for example, could compromise predator evasion (Chopin and Arimoto, 1995; Ryer, 2004) or the ability to control or arrest descent through the water column (Suuronen, 2005). In brook trout (*Salvelinus fontinalis*) subjected to simulated angling stress, subsequent critical swimming performance, U_{crit}, was reduced 75% when angling stress was followed by 2 minutes of air exposure (Schreer et al., 2005). Swimming performance was also compromised in striped bass (*Morone saxatilis*) subjected to severe confinement stress, with the degree of impairment increasing with the increased duration of the stress (Strange and

Cech, 1992). Juvenile walleye pollock and sablefish (*Anoplopoma fimbria*) subjected to simulated trawl capture exhibited reduced swimming speeds and shoal cohesion when threatened by adult sablefish or lingcod (*Ophiodon elongates*), and were consequently more vulnerable to predation (Ryer, 2002; Ryer et al., 2004).

Traditional stock assessment calculations assume that all fish that escape or are discarded from fishing gears survive, despite the growing evidence that mortality in these fish can be extensive (Harley et al, 2000; Suuronen, 2005; Diamond and Campbell, 2009). In addition, the fitness of fish that survive may be reduced through changes in reproductive behaviour and function, growth suppression, altered social function and reduced immunocompetence (fish that do not die as a result of capture-related stresses may suffer impairments in their growth and/or reproduction (Wedermeyer et al., 1990; Barton and Iwama, 1991; Barton, 1997; Suuronen, 2005). Failure to account for the mortality or compromised physiology of juvenile and non-target fish may result in significant underestimation of the impacts of fishing activities on stocks, and undermine management plans (Harley et al., 2000; Diamond and Campbell, 2009). Further, if the survival of escapee fish is low, the benefits of changes to gear selectivity may be overestimated (Suuronen, 2005).

1.6 Snapper, *Pagrus auratus*

Snapper (*Pagrus auratus*, Foster 1801; Sparidae; Fig. 1.6) is one of New Zealand's most important finfish species, representing an iconic recreational and customary catch, as well as a significant commercial fishery (Mossman, 2008; Ministry of Fisheries, 2009).

1.6.1 Snapper biology

Snapper are a demersal fish that occupy a range of habitats, including harbours, estuaries, reefs and open coastline. Although they may be found at depths of up to 200 m, they are most abundant between 15-60 m (Mossman, 2008; Ministry of Fisheries, 2009). Snapper are

Figure 1.6. A juvenile snapper, *P. auratus*, approximately 150mm fork length. Photo courtesy of Matt Walters

widely distributed throughout the warmer waters of Australia and New Zealand; in New Zealand, they are most abundant around Northland, the Hauraki Gulf and the Bay of Plenty, where they are the dominant fish in inshore communities. They are opportunistic feeders, preying on crustaceans, polychaete worms, shellfish, echinoderms and small fish (Mossman, 2008).

Snapper are a slow-growing, long-lived species; they have low rates of natural mortality and may live to 60 years. Snapper first reach sexual maturity between 20-28 cm (3-4 years; Ministry of Fisheries, 2009). Despite being prolific breeders – a 50 cm female may lay 100,000 eggs in a single batch – temperature is a key determinant of spawning success, as well as the growth and survival of larval and juvenile fish (Mossman, 2008). The more southern fisheries, including Hawkes Bay, South Taranaki and Tasman, tend to experience shorter and less reliable spawning seasons; in combination with their slow-growing life strategy, this can render these stocks vulnerable to the effects of overfishing (Mossman, 2008).

1.6.2 Snapper fishery

The snapper fishery is one of the largest and most valuable coastal fisheries in New Zealand (Ministry of Fisheries, 2009). It expanded rapidly in the 1960s and 70s through the

use of trawling (especially pair trawling) and Danish seining. Commercial landings peaked at 18,000 tonnes in 1978, but by the mid-1980s several stocks were exhibiting signs of depletion, and landings fell below 9,000 tonnes. The development of the Japanese "ike jime" market about this time saw the development of the long-line fishery, for its ability to yield higher quality fish (Pankhurst and Sharples, 1992; Ministry of Fisheries, 2009).

As a QMS-managed species, the snapper fishery is divided into several stocks to represent geographic variation in the status of these stocks (Fig.1.7). The present annual Total Allowable Catch (TAC) is 10,663 tonnes; of this, 6,357 tonnes are allocated to commercial fishers. The remainder is designated for allocations within various recreational and customary fisheries (combined 3665 tonnes), and an allowance for illegal fishing, misreporting and fishing-related mortality (611 tonnes) (Ministry of Fisheries, 2009; Ministry for Primary Industries, 2014). The commercial catch is taken predominantly by trawling and longlining, although small catches are also taken by Danish seine and set netting (Forest and Bird, 2009; Ministry of Fisheries, 2009; Ministry for Primary Industries, 2013*a*). More than 50% of this catch is subsequently exported, primarily to Australia, the United States and Japan, at a value of $36 million (Forest and Bird, 2009; Statistics New Zealand, 2009).

In 2013, stock assessments suggested that the snapper SNA1 (East Auckland) stock – a key commercial, recreational and customary fishery – was below biomass targets, and that estimates of the recreational catch for the 2011/2012 season exceeded QMS allowances by some 1350 tonnes (52%). Consequent management changes included a reduction in the daily catch limit (from 9 to 7) and an increase in the minimum legal size (MLS, from 27 to 30 cm), as well as an increase in the recreational allowance such that stock assessment models were better able to account for the observed rates of exploitation. Although no changes in quota were implemented within the commercial fishery, regulatory changes include the need to record the catch and discard of all juvenile snapper, regulations pertaining to the need to "move on" from grounds yielding high catches of juvenile fish, and an increased observer presence for trawl vessels (Ministry for Primary Industries, 2013*a*, 2013*b*).

Snapper has a tender, white flesh with a sweet, mild flavour. It is a versatile fish, being suitable for preparation and cooking by almost any method, including being served raw as sashimi (Pankhurst and Sharples, 1992; Seafood New Zealand, 2013).

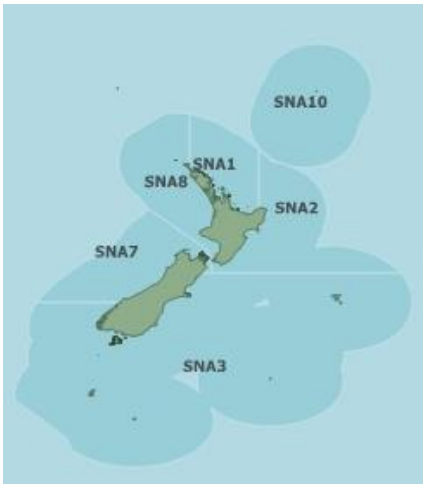


Figure 1.7. Designation of the New Zealand snapper fishery into management stocks

1.7 Thesis objectives

Globally, an increasing demand for fish and fisheries products and growing socioeconomic pressure for the expansion of fisheries are placing significant pressures on wild fish stocks, many of which are already maximally- or over-exploited, and are therefore becoming increasingly vulnerable to the effects of unsustainable fishing practices. As an alternative to increasing landings, improvements in harvesting technologies and practices may offer the potential for industry growth; value and yield can be increased through improvements in quality, reduced waste associated with processing and extended product shelf-life. In addition, improving the sustainability of fishing activities is essential for a number of fisheries where rates of bycatch and subsequent mortality may be disproportionately high. In order to implement targeted improvements in harvesting technologies and practice, however, an understanding of the physiology and response to capture of the target species is crucial.

The snapper (*Pagrus auratus*) is an iconic New Zealand fish species, which yields a well-regarded, often export-quality product. However, given the prevalence of trawl fishing as a means of harvest, opportunities may exist to refine the harvesting process in order to

improve both the quality and/or sustainability of the snapper fishery. The overall objective of this thesis was therefore to gain an understanding of the exercise physiology of snapper, and of the consequences of exhaustive exercise for overall physiological condition. The work has been presented as a number of discrete studies, as outlined below.

Chapter 2: This chapter outlines animal husbandry practices and general methods applicable across the thesis.

Chapter 3: Swimming performance, in particular maximum sustainable swimming capacity, is a key determinant of the interaction between fish and capture gears. The primary aim of this chapter was therefore to characterise the swimming capacity of snapper, as there are no existing measures of swimming performance in this species. In particular, critical swimming performance was used to estimate the maximum sustainable swimming speeds of snapper. In addition, metabolic rate ($MO_{2\ routine}$ and $MO_{2\ max}$) and scope were determined, as was the recovery of MO_2 following fatigue. Allometric relations were determined for both metabolic rate and swimming performance. Similarly, the response of both metabolic rate and swimming performance to temperature acclimation were determined, seeking to mimic the effects of seasonal or geographic variation in temperature.

Chapter 4: The recovery of swimming performance itself following an exhaustive event is an important aspect of exercise physiology, with implications for survival and fitness. This chapter sought to determine the magnitude and duration of any impairment in the critical swimming performance of snapper following exhaustive exercise. Repeat U_{crit} tests were performed following recovery periods of between 0 and 2 hours, in addition to shortened repeat U_{crit} tests, designed to examine more directly the impairment of high-intensity swimming performance.

Chapter 5: Exhaustive exercise is known to result in significant disturbances to metabolic acid-base, haematological and hormonal balance, which have been linked to subsequent changes in post-mortem tissue condition and quality. This chapter sought to characterise the nature and magnitude of such changes in snapper, subject to exhaustive critical swimming tests. The recovery of any disturbance was also determined over a 24 hour period.

Chapter 6: Commercial capture is often considered to be much more stressful for fish than laboratory-based simulations, since fish are exposed to a range of additional stressors. This chapter therefore sought to estimate the validity of using laboratory-based exercise

protocols (i.e. U_{crit}) as a means of simulating trawl capture by comparing the physiological disturbance observed in laboratory-exercised snapper (Chapter 5) with those observed in snapper caught during trawl surveys that mimicked commercial harvesting conditions.

Chapter 7: Environmental oxygen availability is known to be a powerful limiting factor governing the metabolic performance of aquatic organisms. Oxygen limitation may occur naturally within the environment, or locally within trawl nets, with possible consequences for swimming performance. In addition, hypoxia may represent a limiting factor to metabolic recovery following exhaustive capture, both for discarded fish or fish that might be retained within on-board holding facilities where stocking densities are likely to be high. The aims of this chapter were therefore to attempt to characterise the tolerance of snapper to hypoxia, and its possible limitations on swimming and metabolic performance.

Chapter 8: The final chapter discusses the potential implications of the findings of this thesis for improvements in the commercial trawl-based harvesting of snapper, with a view to both improving both product quality and the sustainability of fishing activity. Potential future research directions are also discussed.

CHAPTER 2
General materials and methods

This chapter outlines the general materials and methods used throughout this thesis, including experimental techniques that are common to multiple chapters and fish husbandry practices. Methods specific to a given chapter are detailed within the Materials and Methods section of that chapter.

2.1 Experimental animals

Snapper (*Pagrus auratus*) were obtained from the New Zealand Institute for Plant and Food Research Limited (Nelson, New Zealand) as first-generation fish bred in captivity from wild Tasman Bay broodstock. Snapper were housed indoors in 4.5 m^3 tanks supplied with sand-filtered seawater pumped from Nelson Haven on a flow-through basis (~40 l min^{-1}). Fish were exposed to natural water quality parameters and photoperiod, and ambient water temperature; the geography of Nelson Haven is such that it experiences substantial seasonal variation in water temperature, ranging from approximately 10 ^oC to 22 ^oC (Appendix One). Snapper were fed on a diet of chopped fish, formulated pellets, and a wet mix containing fish, vitamins and minerals, combined in an alginate base.

The majority of the laboratory-based experiments were carried out at the University of Canterbury (UC; Christchurch, New Zealand). To transfer the fish between facilities, fish were initially anaesthetised in 10 or 20 ppm AQUI-S^{TM} (depending on size; see Section 2.5) then dip-netted and transferred to large plastic bags containing a 50% dilution of the initial AQUI-S^{TM} concentration. The bags were sealed, secured within fish-bins, and air-freighted on the Air New Zealand domestic service, before transport by car to the UC aquarium. The

aquarium featured a number of isolated recirculating water systems, each of 1500 l capacity, and containing a biofilter and protein skimmer. Within these systems, the fish were placed into tanks 1.1 m long x 0.9 m wide x 0.6 m high. Water temperature was maintained at 18 $\pm$ 0.2 ^{o}C, salinity at 34 ‰, ammonia below 1.2 mg l^{-1} and nitrite levels below 0.2 mg l^{-1}. Photoperiod was maintained as 12 hours light : 12 hours dark, with a 30 minute "sunset/sunrise" transition between light and dark. Snapper were fed daily on a diet of chopped fish (a firm, white flesh such as warehou) and salmon pellets. Any uneaten food and wastes were removed daily to avoid biological loading.

2.2 Temperature acclimation

All experiments were conducted at a standard acclimation temperature of 18 ^{o}C unless otherwise stated. Where the effects of temperature acclimation on swimming capacity and metabolism were assessed, the additional acclimation temperatures of 12 and 24 ^{o}C were chosen. These temperatures represent the spectrum of temperatures experienced by fish in Nelson Haven, as well as the extremes of temperatures that snapper in their natural coastal environment are likely to experience as a result of geographic and seasonal variations (Majed et al., 2002; Mossman, 2008; Walrond, 2013). It should be noted that the effects of temperature were only assessed following acclimation; no acute temperature exposures were performed during the course of this thesis.

When new fish were received into the UC aquarium, water temperature was set to match the current water temperature at Plant and Food Research; water was then warmed or cooled to the appropriate experimental temperature (i.e. usually 18 ^{o}C) at a maximum rate of change of 2 ^{o}C per day. All other water quality parameters were maintained as described. Once the required temperature was reached, fish were acclimated for a minimum of four weeks before use in experimental work. This also served as an opportunity for fish to recover from transport-related stresses. Resumption of feeding is often used as an indication that fish have recovered from stress (Rice, 1990); food acceptance was always observed within the first week of fish being received.

2.3 Determination of critical swimming speed (U_{crit})

An 80 l Blazka-style swimming flume (Fig. 2.1) was used to assess the critical swimming capacity (U_{crit}) of snapper. The U_{crit} protocol also served as a standardised means by which to exercise the fish and enable the measurement of post-exhaustive changes in physiology, for example, in metabolic rate or tissue biochemistry. The flume consisted of a clear Perspex tunnel (190 mm diameter, 670 mm long) with a fine yet rigid plastic mesh at each end, fitted inside a larger Perspex box (240 mm wide x 270 mm high x 1250 mm long). An impeller at one end of the tunnel generated a water current of known velocity; this velocity could then be manipulated during the course of the experiment.

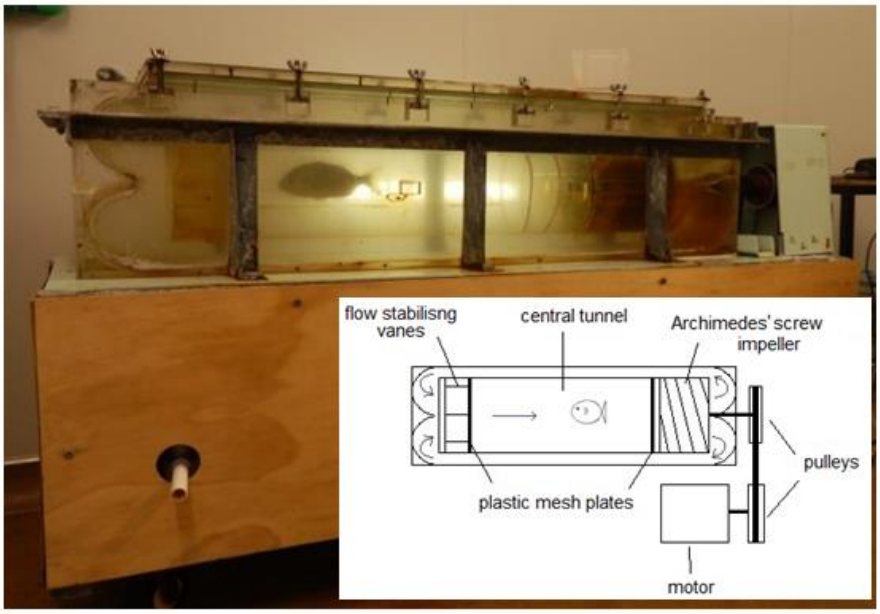


Figure 2.1. The Blazka-style swimming flume used to assess critical swimming speed (U_{crit}) in juvenile snapper. Inset: schematic representation of the flume; the arrows indicate direction of water flow.

U_{crit} trials were performed individually. The fish was placed into the central tunnel of the flume, and the velocity set to 0.5 body lengths per second (bl s^{-1}). A 30 minute "habituation" period was observed to allow adjustment to the light and noise of the flume. The experiment

commenced when the current velocity was increased to 1.0 bl s^{-1}; thereafter, velocity was increased in 1 bl s^{-1} increments at 15 minute intervals, until the fish was exhausted. Exhaustion was defined as the point at which the fish could no longer maintain its position against the current, and became impinged on the rear mesh of the flume (Kolok, 1999). At this point the flume was turned off and the swimming velocity and time of exhaustion were recorded. U_{crit} was determined using the equation

$$U_{crit} = V_p + \left(\frac{T_f}{Ti}\right).V_i$$

where V_p is the penultimate velocity at which the fish swam before exhaustion, T_f is the time elapsed between the velocity increase and exhaustion, T_i is the time interval between increases in velocity, and V_i is the velocity increment (Hammer, 1995).

Several previous studies (Gregory and Wood, 1998; Robinson, 2008) have described removing the fatigued fish from the rear mesh and replacing it into the water current, defining exhaustion as the time at which the fish becomes impinged for the second or third time. During the present study, preliminary experiments found that snapper failed to maintain station soon (<30 seconds) after such replacement, and that it would be appropriate to define exhaustion as the point at which they initially failed to swim.

2.4 Respirometry

2.4.1 Experimental setup

Respirometry is a prominent feature of many of the experiments contained within this thesis. The schematic diagram in Figure 2.2 supports the text in illustrating the experimental setup used for this work. Experiments were set up in an isolated, controlled-environment facility to minimise any disturbance of the fish. A 24 hours light : 0 hours dark lighting regime was employed in an attempt to minimise the effects of any inherent diel variation in oxygen consumption (Fry, 1978; Steffensen, 1989; Appendix Two), and to avoid abrupt changes in light intensity, which appeared to unsettle the fish (personal observation).

All oxygen consumption experiments were conducted using closed-box respirometers constructed from opaque, PVC tube, and with a clear Perspex lid. Respirometers were 1.4 l (150 mm diameter) or 6.7 l (230 mm diameter) for fish smaller or larger than 150 mm in length, respectively. A small submersible pump was used to flush the respirometer with aerated seawater from a 20 l reservoir; both the respirometer and the reservoir were ¾ submerged in custom built water-baths to allow for temperature control. Oxygen partial pressure (PO_2) within the respirometer was measured using a Strathkelvin oxygen electrode and meter (models 1302 and 781 respectively; Strathkelvin Instruments, Glasgow, Scotland), with the oxygen electrode fitted into the lid of the respirometer. The meter was connected to a Powerlab T-26 data acquisition system, with data displayed on a computer using Chart v.7.3 software (both A.D.Instruments, Waverly, N.S.W., Australia).

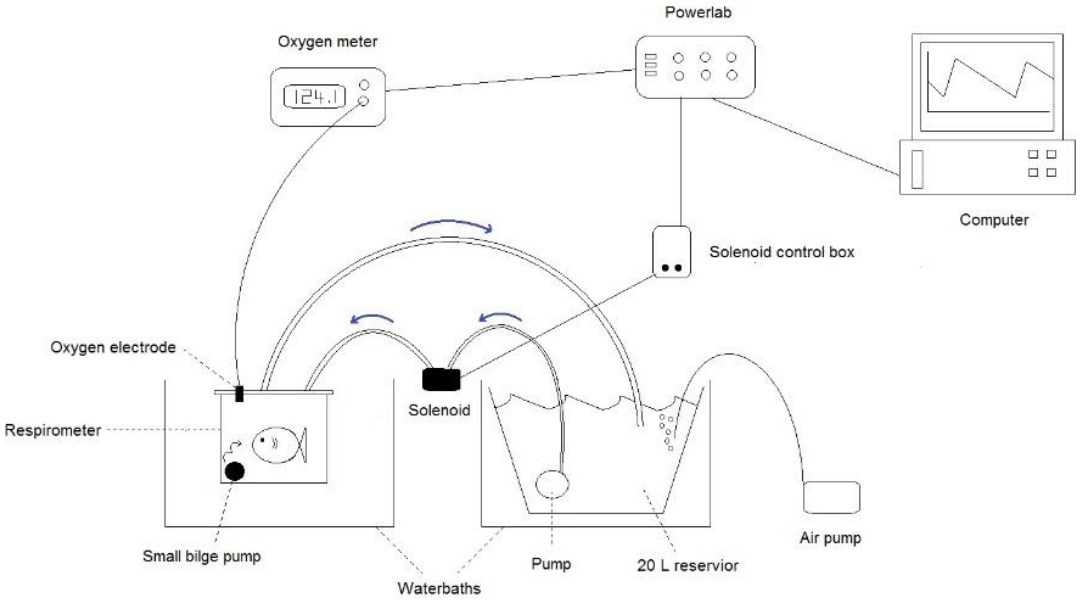


Figure 2.2 Schematic representation of the experimental setup used for respirometry work; arrows indicate the direction of water movement. Oxygen partial pressure was recorded continuously via an oxygen electrode fitted into the lid of the respirometer, and connected via an oxygen meter to the Powerlab data acquisition system. Automation of the system through a Powerlab-solenoid feedback circuit allowed for continuous recording over extended periods and reduced disturbance of the fish, by removing the need for manual flushing of the respirometer.

A solenoid, wired to a simple electrical circuit which was in turn connected to the Powerlab system, was fitted into the tubing between the reservoir and the respirometer to allow the automation of the respirometry system; automation of the system facilitated the continuous recording of MO_2 over an extended period, while minimising the disturbance to fish that may otherwise be associated with the need to manually control the flush cycles. The Fast Response Output function of the Chart software was used to set upper and lower thresholds for respirometer PO_2; when one of these thresholds was reached, an electrical impulse from the Powerlab triggered the solenoid to change its conformation. Thus, the system was set to run such that when the fish depleted the available oxygen to a set PO_2 (the lower threshold), the solenoid would open and allow the respirometer to be flushed with aerated water. When the respirometer had flushed sufficiently (reaching the upper threshold) the solenoid would close, effectively sealing the respirometer and allowing the continued measurement of oxygen consumption. PO_2 thresholds of 120 and 140 mmHg (16.0 and 18.7 kPa) were used to ensure the maintenance of normoxic conditions within the respirometer (Fig. 2.3). Manual override buttons allowed the respirometer to be opened or closed on demand.

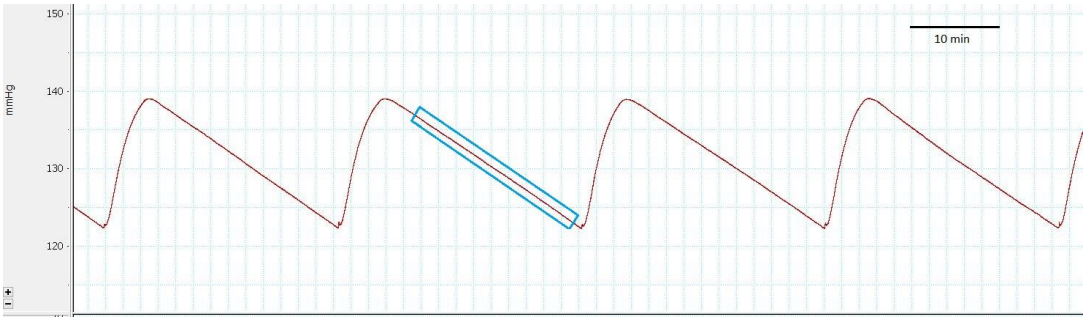


Figure 2.3. Representative recording of respirometer PO_2, demonstrating the oscillation of PO_2 between 120 and 140 mmHg as a result of the automation of the system. MO_2 was calculated by determining the mean rate of change in PO_2 (i.e. the slope of the trace, in mmHg s^{-1}) within the section indicated by the blue frame.

A small galley pump (model 06302, 378 l h^{-1}; TMC Technology Corp., Taiwan) was fitted inside the respirometer. Although is it frequently considered that the routine movements of the opercula or fins, or indeed of the whole animal itself, are sufficient to

circulate the water within a small respirometer (Cech, 1990), preliminary work found that despite the relatively high routine activity of juvenile snapper, the addition of the bilge pump assisted in ensuring the adequate mixing of the water, ensuring uniform oxygen distribution within the respirometer. This resulted in the increased stability of the PO_2 reading, and a smooth, linear fall in PO_2 during periods in which the respirometer was closed. A variable voltage adapter enabled the pump to run at a reduced voltage (3V), reducing its flow rate and preventing undue disturbance of the fish.

Prior to any experimental work, the oxygen electrode was calibrated using an oxygen-free solution of sodium sulphite (20 mg ml^{-1}) and air-saturated seawater, using the equation

$$PO_2 = (P_B - P_{WV}) \times 0.2094$$

where PO_2 is the partial pressure of oxygen in the air-saturated solution (mmHg), P_B is the barometric pressure (mmHg), P_{WV} is the water vapour pressure at the experimental temperature, and 0.2094 represents the molar fraction of oxygen in the atmosphere.

2.4.2 Determination of metabolic rate

Fish were lightly anaesthetised in MS-222 (as described in Section 2.5) and their length and mass recorded. Fulton's condition factor, K, was calculated according to the equation

$$K = \frac{100{,}000M}{L^3}$$

where M is mass (g) and L is fork length (mm). Fish were then placed into individual respirometers.

Fish were set up within the respirometers at least 18 hours prior to the start of any experiment; preliminary work indicated that this was a sufficient period of time to allow for the recovery of stresses associated with handling and anaesthesia (see Appendix Two). Prior to the start of any experiment, the oxygen electrode was calibrated, and any air bubbles gently removed from the respirometer. Care was taken not to disturb the fish during this process; as a precaution, fish were left to settle for 1 hour prior to commencing the

experiment. To begin recording oxygen consumption, the solenoid was closed, sealing the respirometer.

Oxygen consumption (MO_2, µmol g^{-1} h^{-1}) was calculated using the following equation:

$$MO_2 = \frac{\Delta PO_2 \text{ x C x } (V - \left(\frac{M}{1000}\right))}{M \text{ x } \left(\frac{1}{3600}\right)}$$

where ΔPO_2 = the rate of change in PO_2 over the measurement period (mmHg s^{-1}), C = oxygen capacitance of the water at the given temperature (µmol l^{-1} $mmHg^{-1}$), V = volume of water in the respirometer (l), t = time interval between measurements (h), M = mass of the fish (g) (Steffensen, 1989).

In rested fish, oxygen consumption is presented as $MO_{2\ routine}$, being derived from the mean rate of depletion over a 1 hour period. For the determination of $MO_{2\ max}$ and $MO_{2\ post\text{-}exercise}$, oxygen consumption was calculated using $\Delta PO2$ over a 2 minute and 10 minute period, respectively.

To account for any background microbial respiration, a series of blank respirometers were set up as described, though in the absence of fish. The change in PO2 within the sealed respirometer over a 24 hour period was found to be negligible, being equivalent to <1% of the lowest MO_2 observed during this study. To account for any drift in the oxygen electrode, the electrode was calibrated against air-saturated water (as described above) at both the start and conclusion of each experiment. The electrodes were found to be especially stable, drifting less than 2-3 mmHg over a 24 hour period, and hence no correction for drift was applied to the calculation of MO_2.

2.4.3 Flow-through respirometry

Metabolic rate in ~550 g snapper was determined on site at Plant and Food Research, Nelson. An existing flow-through respirometry set-up was used for these measurements, and is detailed below.

Each respirometer consisted of a cylindrical PVC tube (7.4 l, 140 mm diameter, 480 mm length) with clear Perspex ends, mounted on its side and immersed in a 200 l waterbath, where temperature was controlled using a custom refrigeration unit and a GR150 bath heater (Grant Instruments, Cambridge, U.K.). The respirometer was supplied with filtered and UV-sterilised seawater using needle valve taps (SS-R31-4; Swagelok NZ, Auckland, New Zealand) at approximately 800 ml min^{-1}; the exact flow rate was recorded by a FLR1000 Flow Sensor (Omega Engineering, NE, U.S.A.). The temperature of the incurrent water was controlled by passage through an SMO254 stainless steel heat exchanger (WCR, TX, U.S.A.), exchanging with the waterbath. Incurrent and excurrent PO_2 were measured using temperature-compensated Oxyguard Micro oxygen electrodes (Oxyguard, Birkerød, Denmark) mounted anterior and posterior to the respirometer inlet and outlet, respectively. The oxygen electrodes and flow sensors were connected to a Powerlab T-26 data acquisition system, and the data displayed on the computer using Chart v5.2 software to allow continuous recording. A small webcam (Quickcam 900; Logitech, Romanel-sur-Morges, Switzerland), mounted in a custom waterproof housing was submersed in front of the clear face of the respirometer to allow for monitoring of the behaviour of the fish.

Snapper were lightly anaesthetised in 20 ppm AQUI-S^{TM} for approximately 30 minutes, then gently weighed and measured, and placed into respirometers; fish were placed into respirometers 10 hours prior to the start of experimental work (see Appendix Two). Oxygen electrodes were calibrated at the time of setting the fish within the respirometer and again following completion of the experiment, to account for any drift in the electrode; drift (typically <5mmHg) was compensated by adding or subtracting half of the difference between the initial and final calibration values, to the raw data, according to the direction of the drift.

Oxygen consumption (MO_2, µmol g^{-1} h^{-1}) was calculated according to the equation:

$$MO_2 = \frac{\Delta PO_2 \text{ x C x Q}}{M}$$

where ΔPO_2 = difference in PO_2 between incurrent and excurrent water (mmHg), C = oxygen capacitance of the water at the given temperature (µmol l^{-1} $mmHg^{-1}$), Q is the flow of water through the respirometer (l h^{-1}), M = mass of the fish (g) (Steffensen, 1989). No correction was applied for the "washout effect" (Steffensen, 1989; Cech, 1990), since the measurement

of interest – $MO_{2\ routine}$ – was derived under steady-state conditions (including the PO_2 and flow rate of the incurrent water and rates of oxygen consumption). MO_2 was measured over a 24 hour period, calculating the instantaneous MO_2 at 10 minute intervals. $MO_{2\ routine}$ for these fish was defined as the mean MO_2 between 10 and 12 hours post-anaesthesia, as described in Appendix Two.

2.5 Use of anaesthetics

Throughout this thesis, the primary anaesthetic used was the recognised fish anaesthetic, MS-222 (3-aminobenzoic acid ether; Sigma). The dosage varied depending on the nature of the experiment and the size of the fish, and is indicated in Table 2.1. AQUI-S^{TM} was used for the transport of fish from Nelson to Christchurch, and during experimental work carried out at Plant and Food Research.

Table 2.1. Anaesthetic, dosage and exposure times for different size classes of fish and experimental treatments.

	30 g	**75 g**	**150 g**	**600 g**
Transport from Nelson to Christchurch	10 ppm AQUI-S 10, then 5 ppm AQUI-S 10	10 ppm AQUI-S 10, then 5 ppm AQUI-S 10	20 ppm AQUI-S 10, then 10 ppm AQUI-S 10	
Light anaesthesia (measuring mass and length prior to respirometry)	50 mg l^{-1} MS-222 1 min or until most swimming movements ceased	50 mg l^{-1} MS-222 1 min or until most swimming movements ceased	70 mg l^{-1} MS-222 1 min or until most swimming movements ceased	20ppm AQUI-S 10 ~30 minutes until loss of equilibrium
Surgical work (i.e. insertion of ECG electrodes)			80 mg l^{-1} MS-222 2 min until all but opercular movement ceased	
Blood sampling or euthanasia		150 mg l^{-1} MS-222 2 min or until all movement ceased	150 mg l^{-1} MS-222 2-3 min until all movement ceased	

2.6 Ethical consent

The experiments carried out as part of this study were conducted with the approval of the University of Canterbury Animal Ethics Committee, permit number 2009/22R.

2.7 Statistical analysis

The majority of statistical analysis was carried out in GraphPad Prism 5 for Windows (Graphpad Software, Inc., San Diego, U.S.A.). Where analysis required ANCOVA be performed, the statistical package R (www.r-project.org) was used.

All data are presented as mean ± standard error (S.E.) unless otherwise stated. Statistical significance was taken at the level of $p < 0.05$, unless otherwise stated.

Prior to statistical analysis, data were analysed for normality and heterogeneity of variance using the Kolmogorov-Smirnov (KS) test and F-test, respectively. Where one or more of the data sets for comparison failed the KS test for normality, a non-parametric equivalent was used, typically a Mann-Whitney test in place of a Student's t-test. Where data were found to have unequal variance, Welch's correction was applied. Welch's correction was also applied to any comparison where one or more of the data sets had a sample size of <5 (Day and Quinn, 1989).

CHAPTER 3

The effects of body size and temperature on metabolic rate, critical swimming performance and recovery from exhaustive exercise

3.1 Introduction

3.1.1 Metabolic rate of fishes

All organisms require chemical energy to carry out vital cellular functions, with their overall use of chemical energy being termed metabolism (Schmidt-Nielson, 1997; Garrett and Grisham, 2005). The energy expenditure of an organism can be partitioned into two basic levels. The first is associated with essential maintenance and service costs, including the support of respiratory and circulatory systems in providing oxygen and nutrients to tissues; maintenance of ionic and osmotic gradients; production of nitrogenous wastes; and synthesis and turnover of cellular constituents such as lipids and proteins. The second describes the metabolic cost associated with all other "non-essential" activities, including locomotion, digestion and assimilation of food, growth and reproductive development (Brett and Groves, 1979; Cech, 1990; Jobling, 1993).

Metabolic rate is tightly regulated by a complex series of feedback mechanisms, such that the production of ATP is equal to that of demand, avoiding the unnecessary depletion of metabolic substrates (Brett and Groves, 1979; Hochachka and McClelland, 1997; Clarke and Fraser, 2004). In particular, the depletion of ATP and accumulation of metabolic products (ADP, P_i, H^+) signals an increase in metabolic demand, and stimulates an acceleration in the rate of ATP synthesis. Conversely, an abundance of ATP is associated with a reduced demand, and hence the rate of production is adjusted accordingly (Hochachka and McClelland, 1997; Clarke and Fraser, 2004). Metabolic rate is known to be influenced by

species, temperature, season, sex, size, life history, activity level, stress level, nutritional state, reproductive state and ambient oxygen concentrations (Brett and Groves, 1979; Jobling, 1982, 1993; Taylor et al., 2008).

Experimentally, the essential role of oxygen as the terminal electron acceptor during oxidative phosphorylation means measurements of oxygen consumption provide a reliable measure of metabolic rate (Cech, 1990; Jobling, 1993; Schmidt-Nielsen, 1997; Taylor et al., 2008), and hence the terms oxygen consumption and metabolic rate are generally used interchangeably (Schmidt-Nielsen, 1997). It should be noted, however, that such measurements do not account for any anaerobic contributions to metabolism, although these are generally assumed to be minimal under normoxic, routine conditions (Cech, 1990), or that the efficiency of oxidative phosphorylation (i.e. the ratio of ATP produced to ATP consumed) may vary slightly between different metabolic fuels (Kieffer et al., 1998) and under situations of thermal stress (Hilton et al., 2010). Aerobic metabolic rate may be categorised to describe the rate of energy consumption (summarised by Jobling, 1993). Basal metabolic rate is the minimum energy expenditure required to sustain life, through the support of the aforementioned maintenance functions. In fish, the term standard metabolic rate has been adopted in place of basal metabolic rate, as the best measure of basal rates given the constraints associated with experimental conditions. Resting or routine metabolic rates describe the energy consumption of an animal in a quiescent, post-digestive state; routine metabolism is commonly used where fish exhibit a degree of spontaneous activity, as is common in pelagic species. Active metabolic rate is the rate of energy expenditure in animals actively performing behaviours such as locomotion or digestion, with maximum metabolic rate describing the maximum rate at which oxygen can be consumed. Aerobic scope describes the difference between standard and maximum metabolic rates, and therefore represents the capacity to which activities above essential maintenance functions, such as locomotion, digestion, growth or reproductive activities can be supported by aerobic processes (Fry, 1947; Claireaux and Largadere, 1999; Fitzgibbon et al., 2007; Killen et al., 2007; Clarke et al., 2013).

3.1.1.1 Scaling and metabolic rate

Scaling is defined as the structural and functional consequences of a change in size among similarly organised animals (Schmidt-Nielsen, 1977). Many physiological characteristics and rates follow regular trends in accordance with body size, including skeletal mass, body surface area, gill surface area and heart rate and size (Schmidt-Nielsen, 1977). Body mass is one of the most important determinants of metabolic rate, and the general relationship appears universal across taxa: larger animals consume more oxygen than smaller conspecifics under the same conditions; however, on a mass-specific basis, smaller animals have higher rates of consumption. This trend is described by the allometric equation

$$Y = a.X^{b}$$

where Y is the metabolic rate, a is the mass coefficient, X is the mass of the animal, and b is the mass (or scaling) exponent.

Since the works of Ruebner (1883) and Klieber (1932) and the perpetuation of their respective "surface (2/3)" and "3/4" laws, there have been many attempts to explain the fundamental basis for the scaling of metabolic rate and the apparent uniformity of the scaling relationship (i.e. the value of b) across phylogenetic groups. While various models have included considerations pertaining to surface-to-volume constraints, fractal geometry, nutrient and oxygen supply limitations, and ontogenetic changes in relative organ mass (each organ having its own inherent metabolic rate), no one model has been accepted as sufficiently explaining the mechanistic basis for the scaling relationship (Oikawa et al., 1992; West et al., 1999; Dodds et al., 2001; Agutter and Wheatly, 2004; Bokma, 2004; West and Brown, 2005). Several recent reviews of the scaling literature have led some authors to question the validity of universal allometry (Dodds et al., 2001; Bokma, 2004; Glazier, 2005), suggesting instead that there is growing evidence of differential scaling of metabolism among phylogenetic groups. For example, White et al. (2006) suggested mass exponents of 0.68, 0.64, 0.76 and 0.88 for mammals, birds, reptiles and fish, respectively. Other studies have suggested exponents of 0.71 (Bokma, 2004), 0.79 (Clarke and Johnston, 1999), 0.86 (Brett and Groves, 1979) and 0.88 (White et al., 2006) as describing the scaling of metabolism in teleost fish.

The relationship between mass and metabolic rate is further dependent on the nature of the metabolic rate measured. Maximum metabolic rate is generally accepted as having a

higher mass exponent than that of standard metabolic rate. Brett (1965) found that the mass exponent for metabolic rate in sockeye salmon (*Oncorhynchus nerka*) increased in response to increasing levels of activity, from 0.78 at rest to 0.97 during strenuous exercise. Rao (1968) similarly demonstrated an increase in the mass exponent with increasing exercise intensity in rainbow trout (*Oncorhynchus mykiss*). Several additional studies have suggested that the scaling of $MO_{2\ max}$ in fish is approximately isometric (Rao, 1971; Brett and Glass, 1973; Wieser, 1985; Armstrong et al., 1992; Post and Lee, 1996; Killen et al., 2007). The differences in the metabolic scaling of standard and maximum metabolic rates likely reflect the different processes contributing to energy consumption, and that maximum metabolic rate is limited by determinants of aerobic capacity (for example, mitochondrial and capillary density) that may be relatively indifferent to body size (Weibel et al., 2004).

3.1.1.2 Temperature and metabolic rate

Temperature is one of the most pervasive environmental factors influencing aquatic organisms. In fish, intimate contact between the blood and the environment at the gills allows not only for effective gas exchange, but also for heat exchange, and body temperature equilibrates with that of the environment (Jobling, 1994; Taylor et al., 2008). Changes in ambient temperature may therefore be associated with profound changes in the rates of numerous biological processes; increased kinetic energy of cellular constituents at elevated temperatures directly modifies the rates of both physical (i.e. diffusion) and chemical (i.e. enzymatic) processes (Pain, 1987; Schmidt-Nielsen, 1997; Hammill et al., 2004; Taylor et al., 2008; Schulte et al., 2011). The sensitivity of a process to temperature can be described by its Q_{10} value, or the change in the rate of the activity with a 10 ^{o}C increase in temperature.

For poikilothermic animals, changes in temperature may therefore represent a significant metabolic challenge, which can be described by the oxygen and capacity limited thermal tolerance (OCLTT) hypothesis (Taylor et al., 2008; Pörtner, 2010). Within the thermal tolerance range of a fish, metabolic rate typically increases with increasing temperature, with a concomitant increase in oxygen demand. To meet this demand, the cardiorespiratory system augments oxygen delivery via increased ventilatory and cardiac rates and stroke volumes (Heath, 1973; Heath and Hughes, 1973; Taylor et al., 1996; Guderley, 2004; Steinhausen et al., 2008). Ultimately, however, the capacity of the cardiorespiratory system to further

increase oxygen provision to the tissue, and/or the aerobic capacity of the tissues themselves, becomes limiting, such that $\dot{M}O_{2\,max}$, and hence metabolic scope, become impaired (Johnston and Dunn, 1987; Farrell, 2002; Steinhausen et al., 2008; Pörtner, 2010). Limitations in the cardiorespiratory system may be compounded by both a reduction in the solubility of oxygen in the water, and the reduced affinity of haemoglobin for oxygen at increased temperatures. Conversely, at temperatures approaching the lower end of the thermal range, the inability of mitochondria to generate ATP at sufficient rates can also become limiting to performance. This results in a bell-shaped curve for aerobic scope, centred about an optimum temperature where scope is maximal (Pörtner, 2010).

Many species of fish are able to compensate, at least to some degree, the changes caused by temperature to metabolic rate in an attempt to maintain homeostasis and performance of physiological systems. In fish, the acclimation of metabolic rate may take several days to several weeks, depending on the species and the magnitude and rate of temperature change (Jobling, 1994). Acclimatory changes may arise through the modification of membrane composition, mitochondrial and capillary density, cardiac function, the utilisation of metabolic substrates, or changes in the expression of certain enzyme isoforms (Johnston and Dunn, 1987; Sidell and Moerland, 1989; Jobling, 1994; Schmidt-Nielsen, 1997; Guderley, 2004).

3.1.2 Swimming performance in fish

Swimming performance is considered to be one of the main determinants of fitness and survival in fish. During daily routines, swimming performance plays a vital role in the success of essential activities, including foraging and prey capture, predator avoidance, migration, reproduction, schooling and other social behaviours (Videler, 1993; Kieffer, 2000; Plaut, 2001; Claireaux et al., 2006; Hanna et al., 2008*a*; Oufiero and Garland, 2009). Further, in some species, swimming performance may determine the outcome of anthropogenic interactions, such as the negotiation of fish passes and culverts by migrating anadromous fish (Jones et al., 1974; Jain et al., 1998; Peake, 2008) or the evasion of fishing gears (Wardle, 1993; Suuronen, 2005; Winger et al., 2010). Swimming performance may be influenced by the size, sex, reproductive status, nutritional status of the fish, and by the presence of disease or infection, as well as the temperature, salinity, ambient light conditions, oxygen saturation,

and contaminant load of the environment (see reviews by Beamish, 1978; Hammer, 1995; Kieffer, 2000). As sustained locomotor performance is determined by the interaction of multiple physiological systems, including the muscular, cardiorespiratory and nervous systems, it is often considered to be an integrated measure of an animal's physiological capacity for activity within a particular environment (Nelson, 1989; Nelson et al., 2002; Petersen and Gamperl, 2010).

Critical swimming speed, U_{crit}, is a concept coined by Brett (1964) to allow the estimation of the maximum sustainable swimming speed of fish within the laboratory. It involves the forced swimming of fish in an exercise flume, with water velocity increased in set increments and at set time intervals until the fish is fatigued. Despite a wealth of literature on the critical swimming performance of fish (see Beamish, 1978 for a compendium), the functional limits to performance have yet to be fully resolved (Claireaux, et al., 2006; Taylor et al., 2008). It is generally considered, at least for relatively athletic fish species, that aerobic scope limits sustained swimming performance, and the inability of the cardiorespiratory system to further increase oxygen delivery to working tissues results in the recruitment of glycolytic musculature, culminating in fatigue (Beamish, 1978; Farrell, 2002, 2007*b*; Steinhausen et al., 2008).

3.1.2.1 Scaling and swimming performance

Size is amongst the most important of biological constraints on swimming performance (Beamish, 1978; Hammer, 1995; Kieffer, 2000; Mateus et al., 2008). Larger fish are capable of attaining higher swimming velocities – both burst and sustained – than are smaller fish; however, relative performance shows differing size-dependence based on the mode of locomotion employed. When expressed as a function of body size (i.e. body lengths per second, bl s^{-1}), maximum sustained swimming speed decreases with size. For example, Brett and Glass (1973) found that U_{crit} in sockeye salmon was reduced from 4.5 to 2.0 bl s^{-1} as fish increased in length from 10 to 90 cm. In salmonids, the relationship between U_{crit} and size has been described as critical swimming speed being proportional to length$^{0.4}$ (Fry and Cox, 1970), length $^{0.5}$ (Brett, 1965) and length$^{0.63}$ (Brett and Glass, 1973). In contrast, maximum burst swimming velocity has been reported to be independent of size in a number of species, including sockeye salmon (Brett, 1965), herring (*Clupea harengus*; Blaxter and Dickson,

1959), and dace (*Leuciscus leuciscus*; Bainbridge, 1960). However, Bainbridge (1960) noted scaling exponents of 0.58 and 0.65 in rainbow trout and goldfish, respectively, while Wardle (1975) noted (although did not specify fish size), that "small" fish seemed capable of burst speeds of up to 25 bls^{-1}, while "large" fish seemed incapable of speeds beyond 4 bls^{-1}.

The decline in relative performance presumably reflects the disproportionate increase in the power requirements associated with a given swimming speed as fish size increases (Videler, 1993). In addition to the increased drag associated with an increase in body size, drag increases in proportion to (swimming speed)$^{2.8}$ (Bone et al., 1995); thus for larger fish, a given relative speed is defined by a higher absolute speed, and hence higher relative power requirements. Beamish (1978) suggested that burst speed was size-independent by virtue of the increased muscle mass of larger fish being sufficient to meet the costs associated with relative performance. However, other studies have suggested that the burst performance of larger fish may be limited by a decline in the myosin ATPase activity within the white muscle, and in hence maximum contraction velocities and tailbeat frequencies of larger fish (Barany, 1967; Wardle, 1975; Witthames and Greer-Walker, 1982). Critical swimming performance will be constrained in larger fish as a result of the increasing costs associated with relative performance, relative to aerobic capacity, which decreases with size (Post and Lee, 1996).

3.1.2.2 Temperature and swimming performance

The effects of temperature on swimming performance have been well-studied, with the thermal sensitivity of performance showing considerable variation, depending on the species, magnitude of temperature change and mode of swimming being assessed (Beamish, 1978; Hammer, 1995; Kieffer, 2000). Sustained and critical swimming performance may demonstrate pronounced changes in response to both acute and chronic changes in temperature, which are typically described by a thermal performance curve; swimming performance increases with increasing temperature to peak performance at an optimum temperature, T_{opt}, above which further elevations in temperature constrain performance (Fry and Hart, 1948; Brett, 1964, 1967, 1971; Griffiths and Alderice, 1972; Brett and Glass, 1973; Taylor et al., 1996; Lee et al., 2003*c;* although note Jones et al., 1974 found performance to be temperature-independent). Swimming performance may increase several-fold over the

thermal range of a species; for example, in sockeye salmon, U_{crit} increased from 2.5 to 4.5 bl s^{-1} as temperature increased from 1 to 15 ^{o}C, and smallmouth bass (*Micropterus dolomieu*) swam between 2.2 to 14.2bls^{-1} at temperatures ranging between 2 and 30^{o}C (Larimore and Durever, 1968). In contrast, burst swimming performance is often considered to be independent of temperature (Beamish, 1978; Sidell and Moerland, 1989), with Blaxter and Dickson (1959), Beamish (1966) and Jones et al. (1974) all reporting a lack of appreciable change in burst speeds over a range of ecologically relevant temperatures.

The differing thermal sensitivities of different locomotory styles are presumed to result from the different mechanics that underlie each mode of swimming (Beamish, 1978; Bennett, 1990). Sustained swimming performance is constrained by aerobic metabolic scope, with the capacity to provide oxygen to the working musculature itself demonstrating pronounced changes in response to temperature variation (Brett, 1971; Farrell, 2002; Clark et al., 2013). In contrast, Sidell and Moerland (1989) suggest that the contractile function of the WM is ultimately limited by the capacity of glycolytic enzymes to produce ATP, and that these reactions may be relatively temperature-insensitive.

3.1.3 Objectives

Aerobic metabolic scope represents the metabolic limits within which all aerobically-fuelled activities additional to those involved in basal, maintenance functions must be undertaken. An understanding of how metabolic rate and scope vary in relation to biological and environmental factors can therefore provide insight into the ability of an animal to function in different environmental contexts. In particular, aerobic scope may set an upper limit to sustained swimming performance, which is a key determinant of ecological fitness. To date there have been few measurements of metabolic rate or scope in the snapper, *Pagrus auratus* (Cook et al., 2011, 2013; Patel, 2011; Cook and Herbert, 2012), and there is no information regarding swimming capacity in this species. The aim of the present study was therefore to determine the swimming capacity of juvenile snapper, through the use of U_{crit} tests, in addition to measures of metabolic rate and scope. Allometric variation in both swimming performance and metabolic rate were characterised, and the potential influence of temperature on scope and performance (i.e. the OCLTT hypothesis) was also investigated.

3.2 Materials and Methods

3.2.1 Maximum sustainable swimming capacity

Maximum sustainable swimming speed was estimated using an incremental exercise test to determine the critical swimming speed (U_{crit}), as described in Section 2.3. Exercise tests were performed individually. Prior to each test, the Blazka-style swimming flume was filled with aerated water at the relevant experimental temperature. Fish were placed in the central tunnel of the flume for a 30 minute habituation period, with water velocity set at 0.5 bl s^{-1}. The protocol commenced with the increase of water velocity to 1.0 bl s^{-1}; thereafter, velocity was increased in 1.0 bl s^{-1} increments at 15 minute intervals, until the fish was exhausted. Exhaustion was defined as the fish failing to maintain station in the current, and being swept back and impinged on the rear mesh of the flume. The flume was turned off, and the speed and time at the point of exhaustion noted.

3.2.2 Routine metabolic rate, $MO_{2\ routine}$

Fish were lightly anaesthetised in MS-222 (see Section 2.5), and their length and mass recorded. Fish were then placed into individual respirometers, as described in Section 2.4, a minimum of 18 hours prior to the start of oxygen consumption experiments. Following calibration of the oxygen electrode and a subsequent 1 hour precautionary "re-settling" period, experiments commenced when the solenoid was closed, sealing the respirometer. The fall in oxygen tension was recorded in Chart v7.3 over a minimum period of one hour; this change was then used to deduce the animal's routine metabolic rate, $MO_{2\ routine}$. Fast Response Output PO_2 thresholds were set at 120 and 140 mmHg, ensuring the maintenance of normoxic conditions throughout the duration of the experiment. The time taken for PO_2 to decline from 140 to 120 mmHg varied between 10 and 60 minutes, depending on fish mass, respirometer size and temperature.

3.2.3 Maximum and post-exhaustion metabolic rates, $MO_{2\ max}$ and $MO_{2\ post\text{-}exercise}$ and aerobic metabolic scope

Following determination of routine metabolic rate, fish were subjected to a U_{crit} exercise test as described above. Immediately following exhaustion, the fish was removed from the flume and returned to the respirometer; air bubbles were removed and the respirometer sealed, a process that took approximately 1 minute. As per routine metabolic rate, PO_2 was recorded in Chart, using upper and lower PO_2 thresholds of 140 and 120 mmHg respectively. Recovery of oxygen consumption was monitored over the following 24 hour period, with $MO_{2\ post\text{-}exercise}$ calculated at 15 minute intervals for the first 2 hours and half-hourly thereafter.

Maximum metabolic rate, $MO_{2\ max}$, was determined to be the highest MO_2 value for each individual fish, and was always observed within the first two minutes of post-exercise recording.

Aerobic metabolic scope may be expressed as two separate metrics, both of which were calculated for individual fish: absolute scope ($MO_{2\ max} - MO_{2\ routine}$) and factorial scope ($MO_{2\ max} / MO_{2\ routine}$).

3.2.4 The effects of scaling on swimming performance and metabolic rate

To investigate the effects of fish size on swimming performance and metabolism, U_{crit}, $MO_{2\ routine}$, $MO_{2\ max}$ and $MO_{2\ post\text{-}exercise}$, were measured in fish of different size classes. $MO_{2\ routine}$ was measured in 72 fish, ranging in size from 16-626 g. Note that for snapper ~550 g, MO_2 was determined using the flow-through respirometry set-up described in Section 2.4.3. U_{crit} was determined in 129 fish, sized between 94-197 mm (16-157 g). U_{crit} could not be determined for fish larger than 200 mm in length, as their swimming performance exceeded the velocity range of the flume; thus, measurements of $MO_{2\ max}$ and $MO_{2\ post\text{-}exercise}$ were also restricted to measurement in fish 16-157 g, and were determined for 40 individuals. All experiments were carried out at 18 ^{o}C.

3.2.5 The effect of temperature on swimming performance and metabolic rate

To assess the effects of acclimation temperature on swimming performance and metabolic rate, U_{crit}, $MO_{2\ routine}$, $MO_{2\ max}$ and $MO_{2\ post\text{-}exercise}$ were assessed in fish acclimated to one of three temperatures: 12, 18 or 24 ºC. Both the respirometry and swimming components of the experiments were carried out at the relevant acclimation temperature; note that there were no acute temperature exposures performed during this study. The acclimation process itself is described in Section 2.2. To investigate the potential interaction between size and temperature in their effects on swimming performance and/or metabolism, the effects of acclimation were assessed in fish approximately 30 g and 140 g (for each size-temperature group, n = 8; see Table 3.1 for further details).

3.2.6 Statistical analysis

The relationship between the mass and length of fish was determined by fitting an exponential growth curve. The condition factor of the different size-temperature groups were compared using one-way ANOVA.

3.2.6.1 Allometric effects

The effects of fish size on U_{crit} were analysed using linear regression analysis. The relationships between mass and routine and maximum metabolic rates were similarly analysed using regression analyses, following log transformation to linearise data. The resulting regression equations

$$\log(MO_2) = \mathrm{b}.\log(\mathrm{mass}) + \log(\mathrm{a})$$

were then used to calculate allometric equations in the form

$$MO_2 = \mathrm{a}.\mathrm{M}^{\mathrm{b}}$$

where b represents the scaling exponent.

3.2.6.2 Temperature effects

The effects of temperature on U_{crit}, $MO_{2\ rest}$, $MO_{2\ max}$, and metabolic scope, and its potential interaction with size, were analysed using two-way ANOVA. Bonferroni post-hoc analyses were employed where significant results were indicated, to discern which groups were different from each other. To describe the effects of temperature on performance, Q_{10} was calculated using the equation

$$Q_{10} = \left[\frac{R_2}{R_1}\right]^{\left[\frac{10}{T_2 - T_1}\right]}$$

where T_1 and T_2 represent the two temperatures at which performance was determined, and R_1 and R_2 represent the rate or measure of performance at temperatures 1 and 2, respectively.

3.2.6.3 Post-exercise recovery of MO_2

$MO_{2\ routine}$ and $MO_{2\ max}$ were compared for individual fish using Student's t-test of paired design. Similarly, the time taken for $MO_{2\ post\text{-}exercise}$ to return to pre-exercise rates was estimated by the pair-wise comparison of $MO_{2\ routine}$ with $MO_{2\ post\text{-}exercise}$ at subsequent sampling times, using a two-tailed Student's t-test of paired design (Scarabello et al., 1991, 1992). This approach was adopted in preference to ANOVA with post-hoc analysis, since the adjustments in p-values associated with multiple corrections of this nature would have resulted in an unacceptable risk of Type II error (Perneger, 1998).

Excess post-exercise oxygen consumption (EPOC) was determined by calculating the area bound by the $MO_{2\ post\text{-}exercise}$ curve and $MO_{2\ routine}$, between the time of exhaustion and the time at which $MO_{2\ post\text{-}exercise}$ is equal to $MO_{2\ routine}$ (Lee et al., 2003*a*,*c*).

In all comparisons, statistical significance was taken at the level $p < 0.05$.

3.3 Results

The relationship between the mass and length of juvenile snapper is illustrated in Figure 3.1; mass increased exponentially with increasing length, as described by the equation

$$\text{mass} = 8.522e^{0.01449\ \text{length}}$$

There was no significant effect of size or acclimation temperature on the condition factor of fish (Fig. 3.2; $p = 0.9164$).

The key data detailing the effects of fish size and acclimation temperature on critical swimming performance and metabolic rate of snapper are summarised in Table 3.1. Fish swam using sub-carangiform locomotion, with contributions of the pectoral fins evident at low (0.5 to 1 bl s^{-1}) swimming velocities. At speeds of approximately 4 bl s^{-1}, fish began to exhibit burst activity, which became increasingly prevalent as speeds approached U_{crit}.

3.3.1 Effects of fish size and temperature on U_{crit}

The critical swimming speeds observed for snapper (94 to 197 mm length) exercised at 18 ^{o}C ranged from 0.50 to 0.97 m s^{-1} (3.7 to 7.1 bl s^{-1}). Swimming capacity was significantly correlated with body size; absolute speed increased with increasing fork length (Fig. 3.3a; $p < 0.0001$), while relative (i.e. length-specific) performance decreased (Fig. 3.3b; $p < 0.0001$). The allometric relationship between critical swimming speed and fish size can therefore be described as

$$U_{crit}\ (\text{m s}^{-1}) = 0.003142(\text{length}) + 0.2669$$

$$U_{crit}\ (\text{bl s}^{-1}) = -0.01317(\text{length}) + 6.933$$

where length is measured in mm. Considerable variation in U_{crit} was observed between individuals, evidenced by the low R^2 values obtained during regression analysis.

Swimming performance was significantly different between snapper acclimated to different temperatures ($p < 0.0001$). A thermal performance curve was evident, whereby peak critical swimming speeds were observed in fish acclimated to 18 ^{o}C (Fig. 3.4). In 30 g and 140 g fish acclimated to 12 ^{o}C, U_{crit} was 27 and 17% lower respectively, than in fish of comparable size acclimated to 18 ^{o}C. U_{crit} was also comparatively lower (18%) in 30 g fish

acclimated to 24 ^{o}C, while no significant differences were observed between 140 g fish acclimated to 18 and 24 ^{o}C. Q_{10} values for the thermal dependence of U_{crit} between 12 and 18 ^{o}C were 1.7 and 1.4 and between 18 and 24 ^{o}C were 0.7 and 0.9, for 30 g and 140 g fish respectively. The swimming capacity of the smaller fish therefore seemed more sensitive to changes in temperature, and this was further indicated by a significant interaction between temperature and size in their effects on swimming performance ($p = 0.0383$).

3.3.2 Effects of fish size and temperature on $MO_{2\ routine}$ and $MO_{2\ max}$

There was a significant correlation between mass and $MO_{2\ routine}$ (Fig. 3.5; $p<0.0001$); the rate of absolute oxygen consumption increased with increasing mass, M, while mass-adjusted rates of consumption decreased exponentially with increasing fish size. These trends are described by the allometric equations

$$MO_{2\ routine}\ (\mu mol\ h^{-1}) = 15.35\ M^{0.74}$$

$$MO_{2\ routine}\ (\mu mol\ g^{-1}\ h^{-1}) = 15.35\ M^{-0.26}$$

Similarly, the maximum rate of oxygen consumption, $MO_{2\ max}$, was correlated with mass (Fig. 3.6; $p<0.0001$), with absolute consumption increasing and mass-specific consumption decreasing with increasing size, as follows,

$$MO_{2\ max}\ (\mu mol\ h^{-1}) = 24.15\ M^{0.81}$$

$$MO_{2\ max}\ (\mu mol\ g^{-1}\ h^{-1}) = 24.15\ M^{-0.19}$$

Table 3.1. Summary of swimming performance and metabolic rate in 30, 140 and 550 g snapper acclimated to three different temperatures

	30 g			**140 g**			**550 g**
	12 ºC	**18 ºC**	**24 ºC**	**12 ºC**	**18 ºC**	**24 ºC**	**18 ºC**
Mass (g)	29.8 ± 2.7	26.4 ± 2.2	27.5 ± 2.0	121.7 ± 9.7	137.5 ± 6.3	147.1 ± 6.6	541.6 ± 20.6
Length (mm)	110.7 ± 3.2	107.3 ± 2.6	110.1 ± 2.6	179.4 ± 3.9	186.3 ± 2.3	191.3 ± 3.0	285.0 ± 4.6
$MO_{2\ routine}$ (µmol g^{-1} h^{-1})	2.40 ± 0.15	5.72 ± 0.25	9.96 ± 0.60	1.39 ± 0.08	3.53 ± 0.22	7.93 ± 0.30	2.88 ± 0.19
$MO_{2\ max}$ (µmol g^{-1} h^{-1})	7.28 ± 0.50	13.04 ± 0.21	17.91 ± 0.90	6.48 ± 0.45	10.04 ± 0.71	14.82 ± 0.86	
Absolute aerobic scope (µmol g^{-1} h^{-1})	4.88 ± 0.46	7.32 ± 0.21	7.95 ± 0.93	5.09 ± 0.46	6.51 ± 0.64	6.89 ± 0.89	
Factorial aerobic scope	3.07 ± 0.21	2.31 ± 0.08	1.83 ± 0.12	4.77 ± 0.46	2.89 ± 0.19	1.89 ± 0.13	
U_{crit} (bl s^{-1})	4.48 ± 0.26	6.13 ± 0.24	5.05 ± 0.22	3.67 ± 0.15	4.43 ± 0.14	4.28 ± 0.14	
U_{crit} (m s^{-1})	0.50 ± 0.03	0.66 ± 0.03	0.55 ± 0.03	0.66 ± 0.03	0.82 ± 0.02	0.82 ± 0.03	
EPOC (µmol g^{-1})	20.4	12.6	8.2	25.4	33.8	15.3	

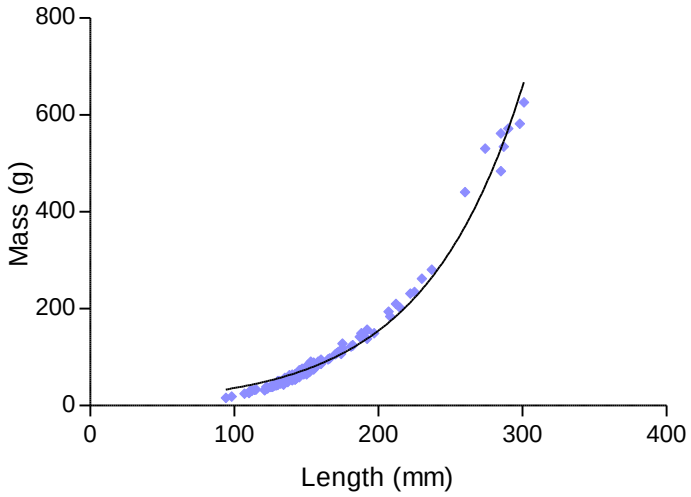


Figure. 3.1. The relationship between mass and length in juvenile snapper. $r^2 = 0.9822$, n = 145.

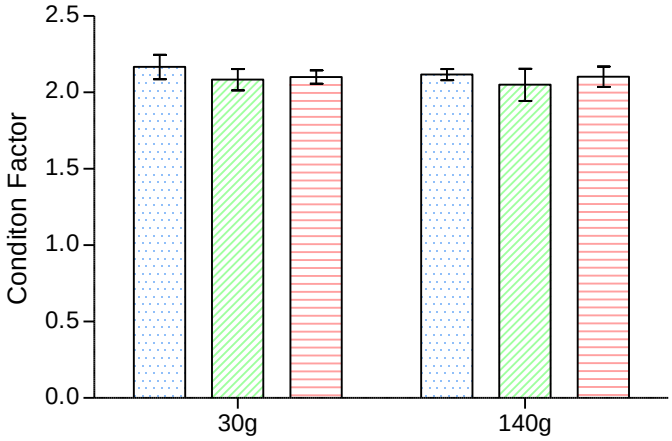


Figure. 3.2. The condition factor of 30 g and 140 g fish acclimated to 12 ºC ( ), 18 ºC ( ) or 24 ºC ( ) for a minimum period of 4 weeks.

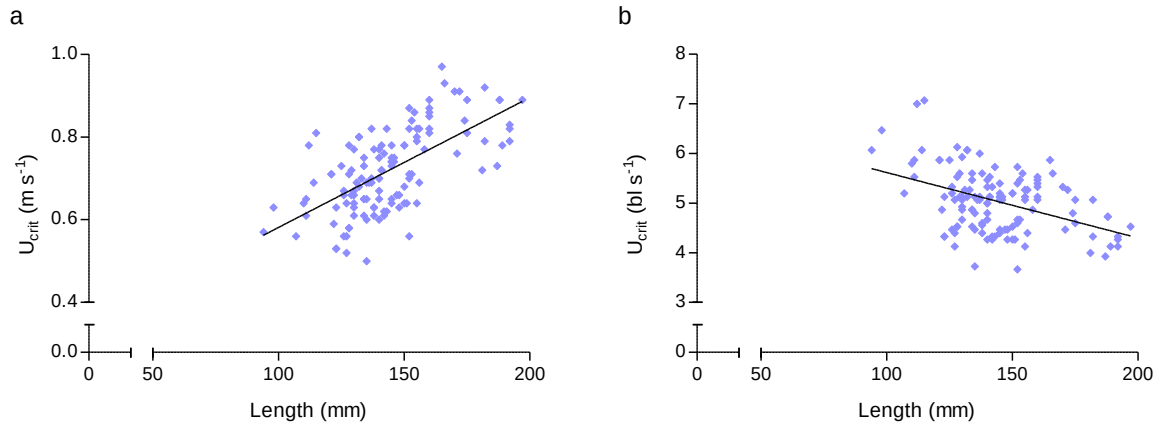


Figure 3.3. Changes in critical swimming speed, U_{crit}, in both a) absolute and b) relative terms, in snapper of different size. Regression equations a) y = 0.003142x + 0.2669, r^2 = 0.4060, p <0.0001; b) y = -0.01317*x* + 6.933; r^2 = 0.1867, p <0.0001.

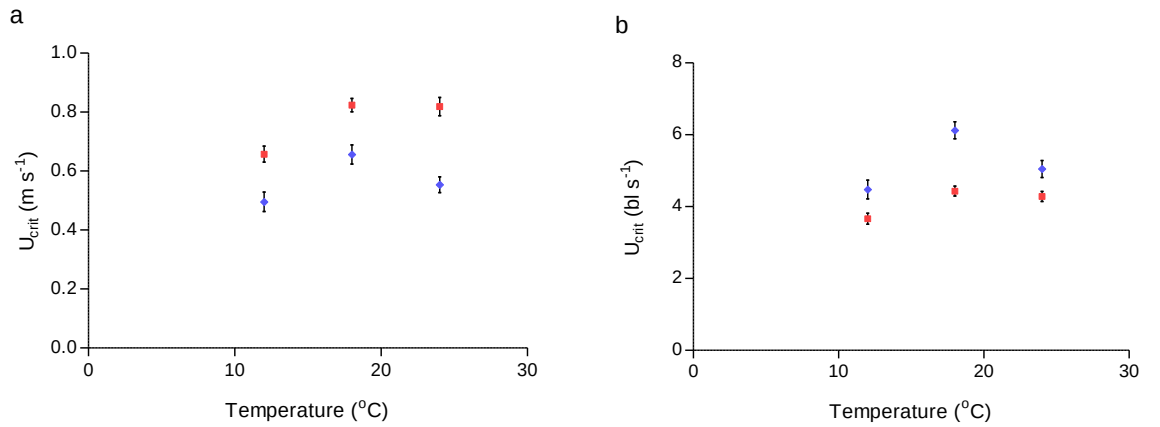


Figure 3.4. Critical swimming speed, U_{crit}, measured in a) absolute and b) relative terms, in 30g (◆) and 140g (■) snapper acclimated to 12, 18 or 24 ^{o}C.

Having accounted for mass-specific differences, there was greater inter-individual variation associated with $\dot{M}O_{2\ max}$ than there was for $\dot{M}O_{2\ routine}$, as evidenced by their respective r^2 value for the log-transformed, linearised plots.

$\dot{M}O_{2\ routine}$ increased exponentially as a function of acclimation temperature (Fig. 3.7), with a Q_{10} of 3.3 in 30 g fish, and 4.3 in 140 g fish. When resolved to smaller temperature brackets, Q_{10} for $\dot{M}O_{2\ routine}$ of 30 g and 140 g fish were 4.3 and 4.7 between 12 and 18°C, and 2.5 and 3.9 between 18 and 24°C. $\dot{M}O_{2\ max}$ also increased exponentially with acclimation to warmer temperatures, although at a lesser rate than did $\dot{M}O_{2\ routine}$ (Fig. 3.7; $p < 0.0001$), with Q_{10} values of 2.1 and 2.0 for 30 g and 140 g fish, respectively. Similarly, when calculated over a smaller thermal range, Q_{10} values were elevated at lower temperatures; Q_{10}s were 2.6 and 2.1 in 30 g and 140 g fish between 12 and 18°C, decreasing to 1.7 and 1.9 between 18 and 24°C.

3.3.3 Effects of fish size and temperature on aerobic metabolic scope

The relationship between mass and aerobic scope was dependent on the metric with which scope was being assessed. Absolute metabolic scope, when considered in mass-specific terms, was highly variable between individuals, and was not significantly correlated with mass (Fig. 3.8a; $p = 0.3176$). When considered as a factorial, however, aerobic scope increased with increasing mass, although the relation was similarly associated with a high degree of inter-individual variation (Fig. 3.8b; $p = 0.0130$).

The relationship between temperature and aerobic scope was similarly dependent on the metric being assessed. Absolute aerobic scope increased as a function of temperature in 30 g fish ($p = 0.0070$), with the rate at which scope increased appearing to slow as acclimation temperature approached 24 °C (Fig. 3.9a). This trend was also suggested in 140g fish, although changes in scope with increasing temperatures were not significant ($p = 0.1719$). In contrast, factorial aerobic scope was significantly reduced with increasing temperature (Fig. 3.9b; $p < 0.0001$), with the changes more profound in 30g than 140g fish.

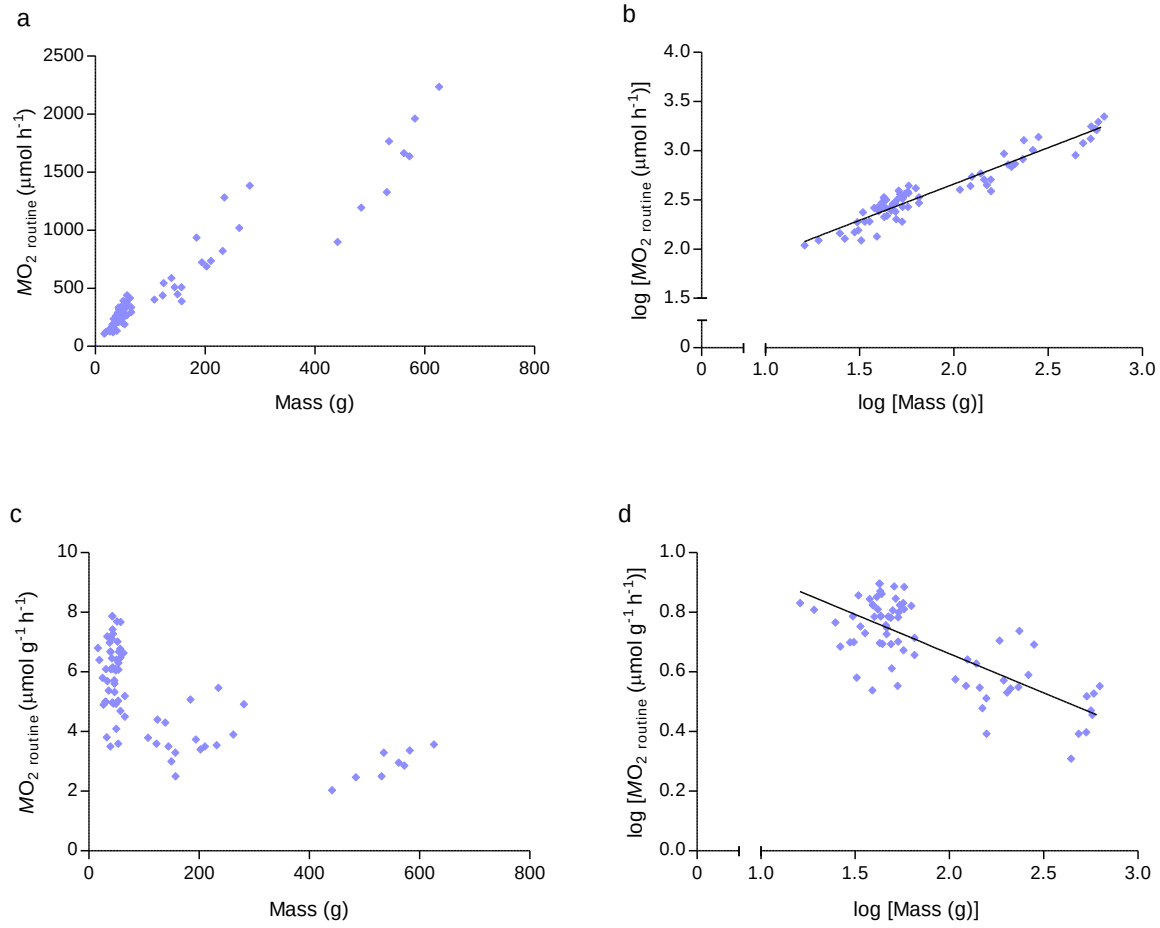


Figure 3.5. Changes in a) absolute and c) mass-specific routine oxygen consumption, $MO_{2\ routine}$, with mass in snapper. Data were log transformed to allow the derivation of allometric equations; regression equations b) y = 0.7377x +1.186, r^2 = 0.9106; d) y = -0.2623x + 1.186, r^2 = 0.5628.

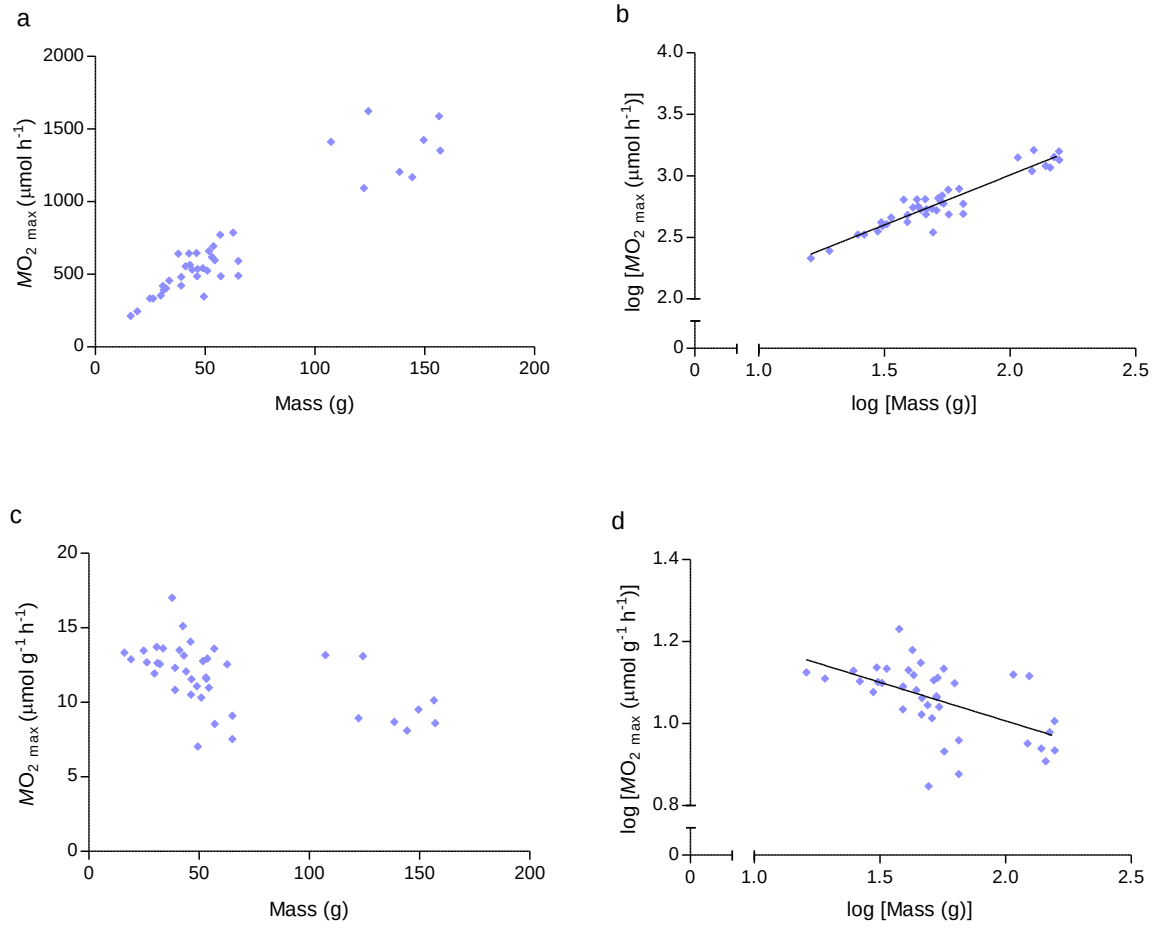


Figure 3.6. Changes in a) absolute and c) mass-specific maximum oxygen consumption, $MO_{2\ max}$, with mass. Data were log transformed to allow derivation of allometric equations; regression equations b) y = 0.8116x + 1.383, r^2 = 0.8858; d) y = -0.1884x + 1.383, r^2 = 0.2948.

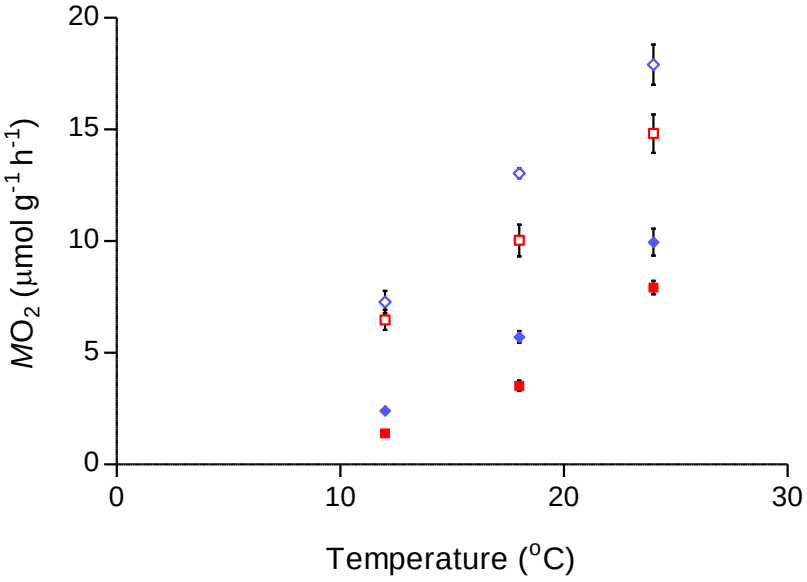


Figure 3.7. Changes in routine and maximum rates of oxygen consumption in 30g and 140g snapper, acclimated to 12, 18 or 24 ºC. ◆ $MO_{2\ routine}$ 30g; ■ $MO_{2\ routine}$ 140g; ◇ $MO_{2\ max}$ 30g; □ $MO_{2\ max}$ 140g.

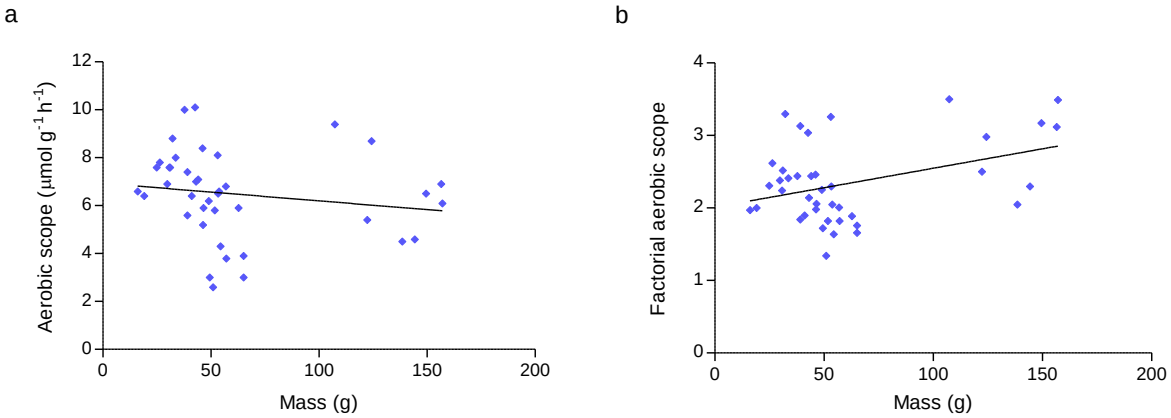


Figure 3.8. Changes in a) absolute and b) factorial aerobic scope with mass in snapper acclimated to 18 ºC. Regression equations: a) y = -0.007333x + 6.932, r^2 = 0.0263; b) y = 0.00536x + 2.011, r^2 = 0.1518.

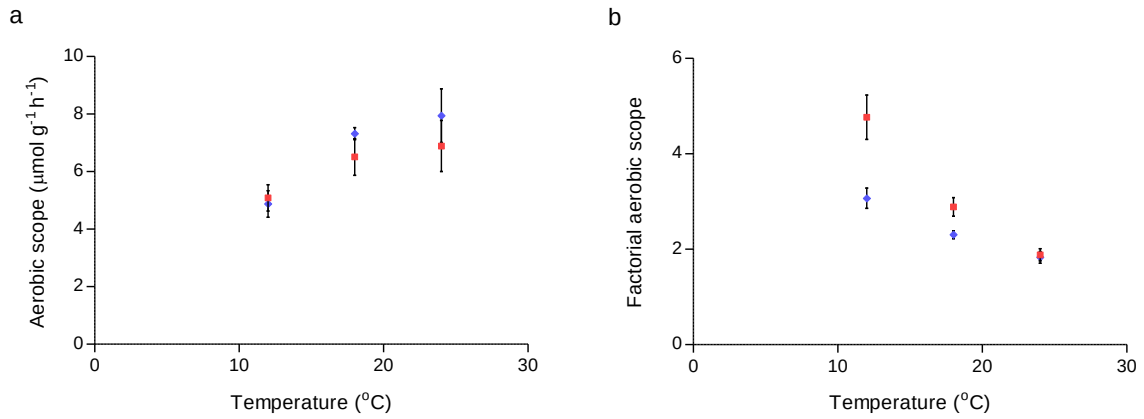


Figure 3.9. Changes in a) absolute and b) factorial aerobic scope in 30g (◆) and 140g (■) snapper acclimated to 12, 18 or 24 ºC.

3.3.4 Effects of fish size and temperature on $MO_{2\ post\text{-}exercise}$ and EPOC

$MO_{2\ max}$ was always observed immediately post-exercise, and thereafter $MO_{2\ post\text{-}exhaustion}$ decayed in an approximately exponential fashion (Fig. 3.10). The period required for the recovery of MO_2 post-exercise appeared to be influenced by both fish size and acclimation temperature: recovery was increasingly rapid with acclimation to higher temperatures, while at comparable temperatures, 30 g snapper exhibited a more rapid return to $MO_{2\ routine}$ than did 140 g fish. The period required for the return of MO_2 to pre-exercise levels in 30 g fish was estimated to be 14.5, 2.5 and 1.5 hours, for fish acclimated to 12, 18 and 24 ºC, respectively. Similarly, the recovery times for 140 g snapper were 17 and 3 hours for fish at 18 and 24 ºC; MO_2 in snapper acclimated to 12ºC did not return to $MO_{2\ routine}$ within the 24 hour recovery period measured. Total EPOC varied between 8 and 24 µmol g^{-1} h^{-1}, depending on fish size and acclimation temperature (Table 3.1). In 30g fish acclimated to 18 and 24ºC, $MO_{2\ post\text{-}exercise}$ appeared to stabilise at a routine level below that determined pre-exercise; recovery times and EPOC determined may therefore be underestimated in these fish.

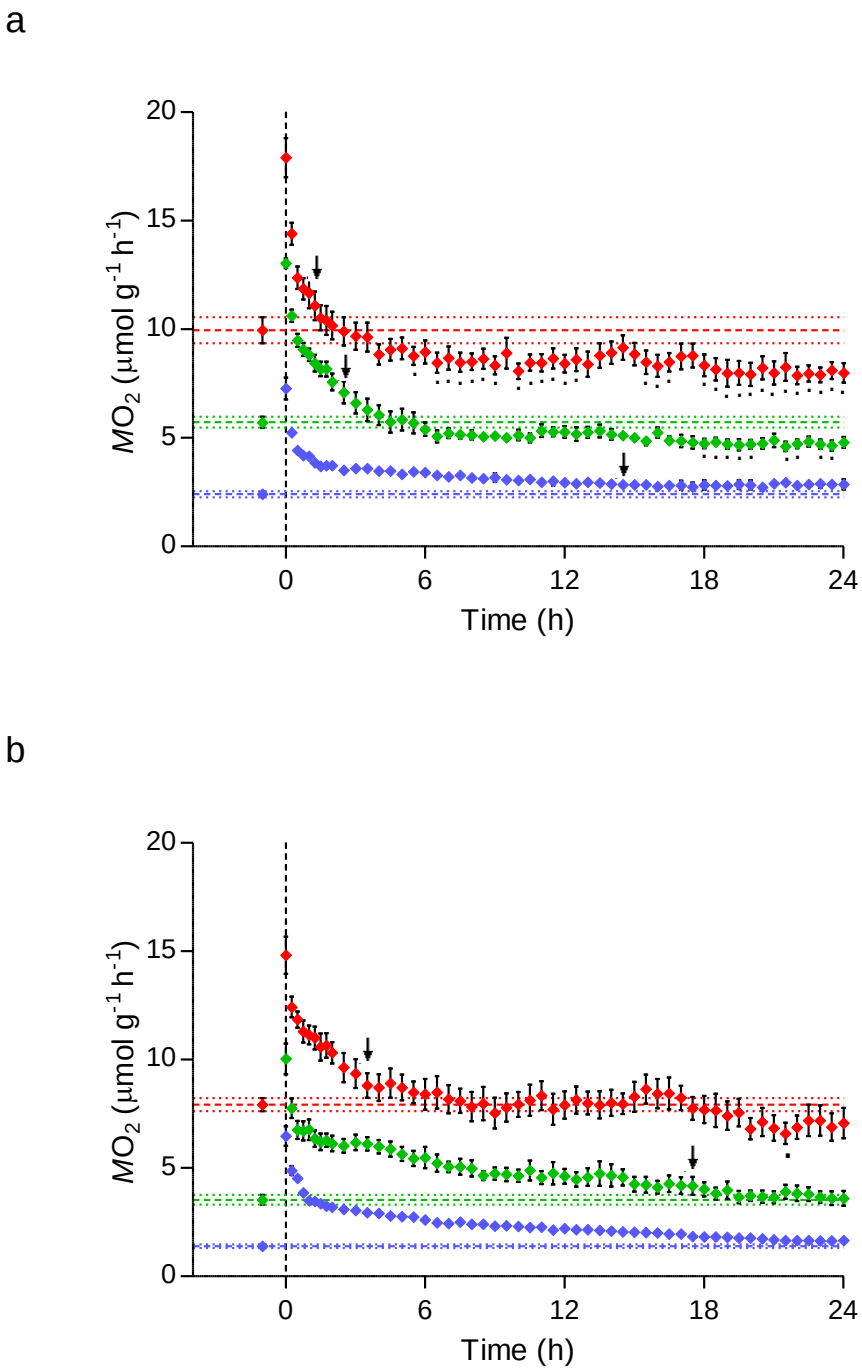


Figure 3.10. Post-exhaustive oxygen consumption in a) 30 g and b) 140 g snapper at three acclimation temperatures (12 ºC; 18 ºC; 24 ºC). The dashed vertical line represents the U_{crit} exercise test. MO_2 values at t = -1 hour represent $MO_{2\ routine}$ for the corresponding temperature, with the mean and standard errors transcribed across the figure for ease of comparison. For clarity, rather than indicate all of the values which are significantly different to $MO_{2\ routine}$, the point at which pre- and post-exercise metabolic rates are no longer significantly different are indicated with an arrow. In fish where $MO_{2\ post\text{-}exercise}$ declined below $MO_{2\ routine}$, significant differences are indicated with a dot below the applicable values.

3.4 Discussion

Metabolism is the physiological engine that powers all cellular processes (Garrett and Grisham, 2005; Fitzgibbon et al., 2007). The measurement of metabolic rate can be useful for describing the physiological state of an organism; in particular, the aerobic metabolic scope – the difference between standard and maximum metabolic rates – represents the capacity to which oxygen uptake may be increased above that required for essential maintenance processes, and thus defines the limits within which all additional aerobic activities must take place. Such constraints ultimately limit sustained swimming performance, an important determinant of ecological fitness in fishes. The present study sought to determine aerobic metabolic scope and swimming performance in snapper, including the potential influence of size and of temperature on performance.

3.4.1 Metabolic rate and scope in juvenile snapper

During the present study, snapper exhibited moderate rates of spontaneous activity whilst within respirometers – typical of pelagic or demersal species – and hence measurements of oxygen consumption under rested conditions are most accurately described as those associated with routine metabolic rate, $\dot{M}O_{2\ routine}$. These routine metabolic rates are in good agreement with the few reported measurements of metabolic rate for snapper of similar size and at comparable temperatures (Table 3.2). Similarly, maximum rates of oxygen consumption were similar to, or exceeded those previously reported for snapper (Table 3.2).

$\dot{M}O_{2\ max}$ is commonly assumed to occur during intense locomotor activity, such as swimming at or near critical swimming speed (Farrell and Steffensen, 1987; Goolish, 1991; Hammer, 1995; Claireaux et al., 2005); however, the suitability of U_{crit} tests as a means of determining $\dot{M}O_{2\ max}$ differs between species, and is reflective of their ecology and lifestyle (Clark et al., 2013). Whilst the aerobic capacity of athletic species has evolved to accommodate the high rates of oxygen consumption associated with exercise, the scope of more sedentary fish has evolved to support other, "more relevant," metabolic costs, such as those associated with digestion (Claireaux et al., 2005; Clark et al., 2013). For example, in Atlantic cod (*Gadus morhua*), crucian carp (*Carassius auratus*), dark barbell (*Peltebagrus fulviaraco*) and southern catfish (*Silurus meridionalis*), the metabolic costs associated with

Table 3.2 Comparison of routine and maximum metabolic rates observed during the present study with those previously reported for snapper.

Mass (g)	Temperature (°C)	$MO_{2\ routine}$ (μmol g^{-1} h^{-1})	$MO_{2\ standard}$ (μmol g^{-1} h^{-1})	$MO_{2\ max}$ (μmol g^{-1} h^{-1})	Reference
30	18	5.72 ± 0.25		13.04 ± 0.21	present study
140	18	3.53 ± 0.22		10.04 ± 0.71	present study
10 – 30	19		5.2 ± 0.3	~ 9.1	Patel (2011)
70 – 50	15		2.7 ± 0.1	~ 7.5	Patel (2011)
200 – 250	18		4.2 ± 0.3	10.6	Cook et al. (2011)
200 – 250	18		4.3 ± 0.4	-	Cook and Herbert (2012)
150 – 300	21	6.2 ± 0.3	4.8 ± 0.4	13.8 ± 0.9	Cook et al. (2013)

digestion and assimilation are additive to those associated with exercise, such that a higher post-U_{crit} MO_2 is attained in fed than unfed fish, indicating that swimming at U_{crit} does not utilise the full aerobic capacity of these species (Blaikie and Kerr, 1996; Dupont-Prinet et al., 2009; Fu et al., 2009; Jourdan-Pineau et al., 2010; Li et al., 2010). In contrast, salmonids yield a similar post-U_{crit} $MO_{2\ max}$ in both fed and unfed fish, and swimming performance is impaired post-prandially, suggesting that $MO_{2\ max}$ is attained at U_{crit}, and hence aerobic capacity must be apportioned between the costs of locomotion and assimilation, at the expense of swimming performance (Alsop and Wood, 1997; Thorarensen and Farrell, 2006). Further, Reidy et al. (1995) found that for Atlantic cod, maximum post-exercise oxygen consumption was dependent on the means by which fish were exhausted, with manual chasing and constant acceleration tests producing 66 and 45% higher $MO_{2\ max}$ respectively, than that produced by U_{crit} tests. These differences were attributed to the possible differential recruitment of anaerobic metabolism during the different exercise regimes, and greater resultant perturbations in metabolic, acid-base and ion balance, which account for a significant proportion of oxygen consumption post-exercise (Scarabello, 1991, 1992; Reidy et

al, 1995). The release of catecholamines, which are known to elevate $\dot{M}O_2$, may also have been more significant during the burst and chase protocols (Reidy et al., 1995).

The similarities in $\dot{M}O_{2\ max}$ elicited by both U_{crit} and chase protocols (Cook et al., 2011; Patel, 2011) suggests that for juvenile snapper, maximum aerobic capacity is recruited during exercise at critical swimming speeds. Investigation of the combined effects of feeding and exercise on metabolic rate and swimming performance would offer the opportunity to confirm this. The similarities in $\dot{M}O_{2\ max}$ between these studies also suggests that any error in $\dot{M}O_{2\ max}$ resulting from the need to transfer fish from the flume to the respirometer following fatigue is likely to be small.

Considerable inter-individual variation in both $\dot{M}O_{2\ routine}$ and $\dot{M}O_{2\ max}$ was observed, despite consistent experimental conditions and having accounted for differences in body mass (Figures 3.5 and 3.6). While such variation in metabolic rate is commonly observed within studies, it has only recently received attention within the literature (Marras et al., 2010; Burton et al., 2011; Norin and Malte, 2012; Bolsden and Malte, 2013). Although it is possible that variation in standard or routine metabolic rate could result from handling stress or experimental error (Steffensen, 1989), there is a growing body of literature demonstrating inter-individual variation in $\dot{M}O_{2\ standard}$ is experimentally repeatable, and is therefore the result of natural, inherent variation between individuals (Cutts et al., 1998, 2001; O'Connor et al., 2000; Marras et al., 2010). The proximate cause of this variation remains unclear, but may include subtle differences in total or relative organ mass, enzymatic activity, or rates of turnover of cellular constituents (Burton et al., 2011). Further, variation in $\dot{M}O_{2\ routine}$, which can be considerable as a result of confounding spontaneous activity, was found to be repeatable in the European eel, suggesting that fish may exhibit specific behavioural patterns that determine the degree of routine activity (Boldsen and Malte, 2013).

In contrast to the observations of Brett (1964, 1965), Forster et al. (1987) and Cutts et al. (2002), inter-individual variation in $\dot{M}O_{2\ max}$ was greater than that associated with $\dot{M}O_{2\ routine}$. That $\dot{M}O_{2\ max}$ typically exhibits less variation between individuals is considered a reflection of the physical limitation of the rate at which oxygen can be delivered to the tissues, whilst standard and routine metabolic rates may be subject to error associated with the extrapolation of $\dot{M}O_2$ or the inherent restlessness and excitability of fish, respectively (Brett, 1965; Jobling, 1994; Cutts et al., 2002). The reason for the greater variation in $\dot{M}O_{2\ max}$ observed for snapper in the present study is unknown, but may reflect individual variation in aerobic capacity.

Alternatively, it may result from variation in the extent of exhaustion attained by fish; several authors have noted a dissociation between metabolic exhaustion and the failure to swim at U_{crit}, suggesting that fatigue may include a behavioural element (McFarland and McDonald, 2002; Peake and Farrell, 2006).

Aerobic scope may be expressed in absolute or factorial terms, representing the absolute and proportionate increases in oxygen consumption respectively, that an animal can achieve above its standard metabolic rate. However, the two metrics often show opposing trends in response to various biotic and abiotic variables, and may therefore yield conflicting conclusions (Clark et al., 2013). Typically, scope is expressed as a factorial, since it provides an accessible index of the change in $\dot{M}O_2$, where absolute measures of scope are arbitrary in the absence of comparative $\dot{M}O_{2\ standard}$ or $\dot{M}O_{2\ routine}$ data. However, Clark et al. (2013) argue that since it takes a specific amount of energy to perform a given activity, rather than a proportionate increase above standard metabolic rate, absolute scope may be a more logical and informative presentation of aerobic capacity. For example, comparison of the factorial and absolute aerobic scopes of pink salmon (*Oncorhynchus gorbuscha*; 3.9 and 16.1 mg min^{-1} kg^{-1}, respectively) and Murray cod (*Maccullochella peelii peelii*; 3.8 and 2.0 mg min^{-1} kg^{-1}) highlights how absolute scope may provide better insight into aerobic capacity. Further, the factorial index is especially sensitive to changes in $\dot{M}O_{2\ standard}$, and may therefore imply a reduced scope even where the capacity for oxygen uptake – as absolute scope – is unchanged. We have therefore heeded the suggestion of Clark et al. (2013) and reported both metrics for the sake of completeness.

While Brett (1964) reported a 10 to 12-fold increase in the rates of oxygen consumption of swimming sockeye salmon relative to fish at rest, factorials in the range of 3 to 7 are more commonly reported (Johnston et al., 1991; Schurmann and Steffensen, 1997; Fitzgibbon et al., 2007; Claireaux et al., 2000; Lee et al., 2003*a*, 2003*b*, 2003*c*). In the present case, factorial scope ranged between 1.8 and 4.8, depending on fish size and acclimation temperature. As an extension of the similarity in $\dot{M}O_{2\ routine}$ and $\dot{M}O_{2\ max}$ to those reported by Cook et al. (2011, 2013) and Patel (2011), both absolute and factorial scope are similar to existing measures of scope for snapper of comparable size and acclimation temperature.

3.4.1.1 Effects of mass on metabolism and aerobic scope

One of the most important determinants of metabolic rate is body mass; that metabolism scales with mass in accordance with the power function $y = aM^b$ is a widely accepted tenet of biology, with the mass exponent, b, reflecting the degree to which mass influences metabolic rate (Brett and Groves, 1979; Xiaojun and Ruyung, 1990; Bokma, 2004; Glazier, 2005; White et al., 2006; Killen et al., 2007). For snapper ranging in size from 16 to 626 g and acclimated to 18 ^{o}C, the mass-dependence of $MO_{2\ routine}$ was described by a mass exponent of 0.74, such that smaller snapper had higher mass-specific metabolic rates than did larger fish. Several reviews of the literature have suggested scaling exponents for $MO_{2\ standard}$ in teleosts ranging from 0.71 to 0.88 (Brett and Groves, 1979; Clarke and Johnston, 1999; Bokma, 2004; White et al., 2006). However, in their review of 69 fish species over 110 studies, Clarke and Johnston (1999) point out that despite a mean scaling exponent of 0.79, 80% of the individual studies included in the review reported exponents between 0.65 and 0.95, with a total range of 0.40 to 1.29. Jobling (1994) similarly concluded that the majority of mass exponents published for fish fall between 0.65-0.9. This disparity in the value of exponents implies variation in the nature of the mass-metabolism relationship between species or families, and argues against a universal allometry for fish (Jobling, 1994; Clarke and Johnston, 1999). There is also evidence to suggest that environmental conditions – especially temperature – may influence the nature of the relationship between mass and metabolic rate, and hence the value of any exponent (Brett and Glass, 1973; Jobling, 1983, 1994; Xiaojun and Ruyung, 1990; Johnston et al., 1991), although temperature-independence of metabolic isometry is also reported (Brett, 1964). Caution should therefore be applied in making direct comparisons of exponents across studies (Jobling, 1993).

Data regarding the scaling of $MO_{2\ max}$ is more sparse, particularly for ectotherms (Killen et al., 2007). The scaling of $MO_{2\ max}$ is not a simple extrapolation from $MO_{2\ standard}$, since different processes contribute to the overall oxygen consumption in each instance; rather, $MO_{2\ max}$ in fish is generally considered to approach isometry, reflective of the limitation of $MO_{2\ max}$ by determinants of aerobic capacity (i.e. mitochondrial or capillary density, gill surface area), which may be relatively indifferent to changes in body size (Weibel et al., 2004; Nilsson and Östlund-Nilsson, 2008). However, since aerobic capacity is often associated with lifestyle, differences in the scaling of $MO_{2\ max}$ are often observed between species of differing athletic capacities. For example, Killen et al. (2007) found $MO_{2\ max}$ in the

benthic ocean pout (*Macrozoarces americanus*) and shorthorn sculpin (*Myoxocephalus scorpius*) and the semi-pelagic lumpfish (*Cyclopterus lumpus*) to scale with mass$^{0.88\text{-}0.92}$, while mass exponents of 0.97, 0.99 and 1.14 have been reported for sockeye salmon and rainbow trout, respectively (Brett, 1965; Brett and Glass, 1973; Weiser, 1985). It is therefore interesting that the mass exponent for $M\mathrm{O}_{2\ \mathrm{max}}$ observed in the present study is so low, given the relatively athletic nature of snapper implied by their critical swimming performance (see Section 3.4.2). The reason(s) for this apparent discrepancy are unclear, but may reflect the relatively small size range for which $M\mathrm{O}_{2\ \mathrm{max}}$ could be assessed (16-157 g) as a result of velocity limitations of the swimming flume. Wieser (1985) and Post and Lee (1996) have stressed the importance of examining fish over a wide size range in order to accurately determine the nature of the relationship between $M\mathrm{O}_2$ and mass. Cutts et al. (2002) also observed a low mass exponent for $M\mathrm{O}_{2\ \mathrm{max}}$ (0.83) in juvenile Atlantic salmon, which was not significantly different to that of $M\mathrm{O}_{2\ \mathrm{standard}}$, and which may have resulted from the small size range included in the study.

There was no discernible relationship between mass and absolute aerobic scope, while factorial scope increased significantly with mass, on account of the disproportionate effect of higher relative $M\mathrm{O}_{2\ \mathrm{routine}}$ in smaller fish. In both cases, however, inter-individual variation in scope was substantial. Allometric differences in scope have been well documented in previous studies, with factorial scope increasing with mass, while absolute scope is reduced (Brett, 1965; Wieser, 1985; Armstrong et al., 1992; Post and Lee, 1996; Killen et al., 2007), although the mass-independence of scope has also been reported (Cutts et al., 2002; Huang et al., 2013). The mass independence of absolute scope observed in the present study suggests that the potential for aerobic scope to limit performance in snapper of different size will depend on the activity concerned (for example, locomotion, assimilation, growth or reproductive activities), and in particular the scaling of any associated costs. For example, the cost of transport – the cost of moving a unit mass a given distance – is inversely related to size (Schmidt-Nielsen, 1972; Videler, 1993); hence the capacity of aerobic metabolism to support locomotion may be reduced in smaller snapper.

3.4.1.2 Effects of temperature on metabolism and aerobic scope

Temperature is one of the most important abiotic factors influencing the physiological function of fish; the equilibration of body temperature with that of the environment has profound consequences for numerous biological processes, should ambient temperature change (Jobling, 1993; Taylor et al., 2008). Fundamental thermodynamic laws dictate that the rates of biological reactions increase with increasing temperature, and the sum of these changes manifests as elevated metabolic rate, with concomitant changes in oxygen consumption (Johnston and Dunn, 1987; Schulte et al., 2011).

$MO_{2\ routine}$ and $MO_{2\ max}$ increased exponentially with increasing acclimation temperature, as has been well-documented for fish (Brett, 1964, 1971; Brett and Glass, 1973; Brett and Groves, 1979; Johnston et al., 1991; Lee et al., 2003*c*). Q_{10} values for $MO_{2\ routine}$ were 3.3 and 4.3 in 30 g and 140g snapper respectively, higher than reported elsewhere for other fish species (with a Q_{10} of between 1.5 and 3 typically reported in both acutely exposed and acclimated fish; Beamish, 1964*a*; Brett and Glass, 1973; Weiser, 1973; Jobling, 1993; Clarke and Johnston 1999; Mallekh and Largadere, 2002; Lefrancios and Claireaux, 2003;). Q_{10} values for $MO_{2\ max}$ were more typical, at 2.1 and 2.0 respectively. The reason for the particular temperature sensitivity of routine metabolism remains unclear, but may reflect the use of routine rather than standard rates, since spontaneous activity may be greater in warm-acclimated fish and therefore inflate the increase in MO_2 beyond that associated with standard metabolic rate (Beamish, 1964*a*; Cossins and Bowler, 1987). Although no direct comparison of activity was made, it was noted that snapper acclimated to 12 ^{o}C appeared more docile within the main aquarium than did fish acclimated to warmer temperatures.

The increase in $MO_{2\ max}$ is ultimately limited by the capacity of the cardiovascular and respiratory systems to further increase oxygen delivery to metabolically active tissues; consequently, aerobic scope is reduced as temperature is further increased, since the increase in $MO_{2\ routine}$ continues at temperatures above those where $MO_{2\ max}$ is limited, before declining as lethal temperatures are approached (Mallekh and Largadere, 2002; Farrell, 2002, 2007*b*; Steinhausen et al., 2008; Pörtner, 2010). In the present case, the relatively small increase in absolute aerobic scope between 18 and 24 ^{o}C suggests that the temperature at which peak aerobic scope is observed, $T_{opt\ AS}$, is imminent, in particular for 140 g fish. Interestingly, the constraint on aerobic scope is evident despite MO_2 appearing to have scope

to increase further, although the reduced Q_{10} between 18 and 24 compared with 12 and 18 suggest that the rate of increase in $MO_{2\ max}$ is also slowing.

Typically, the temperatures at which peak aerobic scope and peak $MO_{2\ max}$ occur are similar. The apparent dissociation between the two during the present study may suggest a disproportionate increase in $MO_{2\ routine}$ at higher temperatures, for example as a result of increased spontaneous activity and agitation, giving the appearance of a constrained scope. If this is the case, $T_{opt\ AS}$ may be slightly higher than that implied presently.

The mechanistic basis for the limitation of $MO_{2\ max}$, and hence aerobic scope, are described by the OCLTT hypothesis with increasing temperature remains somewhat unclear. Despite a reduction in both the solubility of oxygen in water and of the affinity of haemoglobin for oxygen at elevated temperatures, both of which may impair oxygen uptake at the gills (Farrell, 2002; Peck et al., 2004), the arterial blood of salmonids remains saturated during thermal stress, even at the point of cardiovascular collapse, suggesting oxygen limitation at the gill is not a factor in restricting scope (Heath and Hughes, 1973). Alternatively, Farrell (2002, 2007*b*) suggests that limitations to cardiac performance ultimately limit aerobic scope at high temperatures. As the terminal organ in the systemic circulation, the heart relies on the oxygen content of the venous blood, although some species possess a supplementary coronary circulation. Increasing oxygen extraction by somatic tissues at elevated temperature depletes venous oxygen content, which in combination with the increasing costs of cardiorespiratory work required to augment oxygen delivery may result in myocardial hypoxia. Hypoxia is known to impair the contractile function of the myocytes (Steffensen and Farrell, 1998), limiting cardiac output and consequently, aerobic scope.

3.4.2 Swimming performance in juvenile snapper

Critical swimming speed varies substantially across species, reflective of the diverse locomotory requirements of fish with different ecologies (see Beamish, 1978 for a compendium). These are the first reported measurements of U_{crit} for snapper; speeds of up to 7.1 bl s^{-1} attest to a strong swimming capacity in these fish. Although the comparison of swimming performance across studies may be complicated by differences in fish size,

temperature, population history and experimental conditions, examples of swimming performance in fish of differing lifestyles are presented for comparison in Table 3.3. For example, scombrid fish, such as the kawakawa (*Euthynnus affinis*) and chub mackerel (*Scomber japonicus*), are renowned for their ability to sustain high swimming velocities over extended periods (Magnuson, 1978; Sepulveda and Dickson, 2000); schools of skipjack tuna (*Euthynnus lineatus*) have been observed to sustain speeds estimated at 5 to 8 bl s^{-1} for over an hour (Magnuson, 1978). Similarly, salmonids require a high capacity for sustained swimming in order to successfully complete their upstream migrations prior to spawning (Jain et al., 1998). In contrast, fish with more benthic or sedentary habits, such as the Atlantic cod and turbot (*Scophthalmus maximus*), have a considerably lower capacity for sustained exercise. The capacity of snapper to sustain moderate swimming velocities would be consistent with their lifestyle – that of a dermesal fish that may spend considerable time in the open ocean and along coastlines, and as actively foraging predatory fish (Mossman, 2008).

Significant variation in U_{crit} was observed between individuals, despite consistent environmental conditions and having accounted for allometric variation (Fig. 3.3). Although few studies tend to address such variation, individual swimming performance is reported to be experimentally repeatable in a number of species, including rainbow trout (Gregory and Wood, 1998), Atlantic cod (Nelson et al., 1994), and largemouth bass (*Micropterus salmoides*; Kolok, 1992), suggesting that such variation reflects natural, inherent variation within individuals rather than experimental error (Kolok, 1999). A study of repeat swimming performance in snapper, presented in Chapter 4 of this thesis, suggests that individual performance in juvenile snapper is also experimentally repeatable.

Despite the wealth of literature that exists pertaining to the swimming performance of fishes, the functional limits of sustained swimming speed remain unresolved (Jones and Randall, 1978; Taylor et al., 2008). Sustained swimming activity is the net result of the complex integration of metabolic, cardiorespiratory, nervous and muscle functions, and hydrodynamics, with the performance of each having the potential to vary with species, fish size and temperature (Marsh, 1990; Claireaux et al., 2006; Taylor et al., 2008). The lack of integrated studies has made it difficult to determine the relative importance of each in limiting the capacity for sustained locomotion (Jones and Randall, 1982; Claireaux et al., 2006; Taylor et al., 2008). Sustained performance is generally considered to be limited by the capacity of the cardiovascular and respiratory systems to extract and deliver oxygen to the

working musculature, since muscle function during sustained activity must be supported exclusively by aerobic metabolism (Jones and Randall, 1978; Sidell and Moerland, 1989). For example, the swimming capacities of rainbow trout and Chinook salmon (*Oncorhynchus tshawytscha*) are reduced post-prandially, since aerobic scope must be apportioned between the costs associated with both locomotion and assimilation (Alsop and Wood, 1997; Thorarensen and Farrell, 2006). Further, during experimental manipulations in which aerobic scope was functionally reduced, including environmental hypoxia (Jones, 1971; Fitzgibbon et al., 2007; Petersen and Gamperl, 2010; Fu et al., 2011; McKenzie et al., 2012), anaemia (Jones, 1971), and splenectomy (Pearson and Stevens, 1991), critical swimming performance was subsequently impaired. There is some evidence to suggest that limitations to cardiac performance, possibly as a result of oxygen limitation due to its dependence on venous oxygen content, ultimately restrict oxygen delivery to the myotomal muscles (Farrell, 2002, 2007*b*; Simonot, 2006; Steinhausen et al., 2008). In particular, venous oxygen tension (P_vO_2) and cardiac output (Q) plateau prior to U_{crit} in rainbow trout (Farrell and Clutterham, 2003; Simonot, 2006), and coronary ligation impaired critical swimming performance in Chinook salmon (Farrell and Steffensen, 1987). In contrast, however, coronary ligation and splenectomy have been found to have no effect on U_{crit} in squawfish (*Ptychocheilus oregonensis*)(Kolok and Farrell, 1994) and rainbow trout (Gallaugher et al., 1992), respectively, and further, the measurement of oxygen tensions within the RM of swimming rainbow trout revealed a $PO_{2\ RM}$ of 40 mmHg at U_{crit} (McKenzie et al., 2004), suggesting oxygen provision to the RM was not limiting to swimming performance.

While limitations in the provision of oxygen to the tissues would ultimately limit sustained swimming performance, other aspects of locomotor capacity, in particular muscle function, could also be important in determining maximum sustainable swimming velocities (Sidell and Moerland, 1989). As the mechanical power output of the muscles required to overcome drag increases in proportion to (swimming speed) $^{2.8}$, RM fibres are increasingly recruited with increasing swimming speed (Bone et al., 1995). Ultimately, maximum RM recruitment and thus peak power output is achieved – often at only modest swimming speeds (Sidell and Moerland, 1989). WM recruitment may therefore be necessary to supplement the power output required for any further increase in swimming velocity, marking the transition from sustained to prolonged locomotion.

Table 3.3 Critical swimming speed, U_{crit}, of snapper in comparison with other teleost species.

Species	Mass (g)	Length (mm)	Temperature (°C)	Ucrit (bl s^{-1})	Reference
Snapper	16 – 157	94 – 197	18	3.7 – 7.1	Present study
Kawakawa	24 – 265	116 – 255	24	3.4 – 5.1	Sepulveda and Dickson, 2000
Chub mackerel	26 – 156	133 – 250	24	3.8 – 5.8	Sepulveda and Dickson, 2000
Sockeye salmon	50	170	5 – 10	3.0 – 4.2	Brett, 1967
	2000 – 3000	580 – 640	8 – 18	1.6 – 2.1	Lee et al., 2003*a*, 2003*b*, 2003*c*
Rainbow trout	5 – 100	60 – 200	10	5.5 – 6.4	Fry and Cox, 1970
Coho salmon		70 – 100	20	5.8	Griffith and Alderice, 1972
	2300	580	8	1.7	Lee et al., 2003*b*
Brook trout		100	15	4.6 – 5.1	Peterson, 1974
Atlantic cod	79 – 473	210 – 390	5 – 15	1.6 – 1.9	Schurmann and Steffensen, 1997
Burbot	7 – 1100	120 – 620	12	0.7 – 3.0	Jones et al., 1974
Pike	7 – 1800	120 – 620	12	0.8 – 1.6	Jones et al., 1974
Turbot	400 -0600		6 – 22	0.5 – 2.0	Mallekh and Largadere, 2002

Species names not cited in-text: brook trout, *Salvelinus fontinalis*; burbot, *Lota lota*; pike, *Esox lucius*

3.4.2.1 Effects of fish size on swimming performance

Size is amongst the most important of biological constraints on swimming performance in fish (Beamish, 1978; Hammer, 1995; Kieffer, 2000). The scaling relation of critical swimming performance of snapper was consistent with that which has been documented elsewhere for fish – U_{crit} increases with size, but when considered in relative terms, performance favours smaller fish. In fish acclimated to 18 ^{o}C, U_{crit} was reduced almost 30% as fork length increased from 107 mm (~30 g) to 186 mm (~140 g). The calculation of a scaling exponent through the log-transformation of the length-U_{crit} relationship suggests that critical swimming speed is proportional to length$^{0.62}$, similar to the relationship observed in salmonids, where exponents of 0.4 (Fry and Cox, 1970), 0.5 (Brett, 1965) and 0.63 (Brett and Glass, 1973) have been reported.

Swimming performance is the net result of the capacity of the muscle to generate power relative to the resistive forces of the water. In addition to the increase in drag associated with a larger body size, drag also increases in proportion to (velocity)$^{2.8}$; hence the relative performance of larger fish, given their higher absolute speed, requires a greater power output by the muscle (Webb, 1977; Videler, 1993; Bone et al., 1995). During sustained swimming, the capacity of the muscle to meet these requirements will be constrained by aerobic capacity. During the current study, no significant variation in absolute scope – the oxygen available to fuel sustained activity – was observed between fish of different size; hence, the higher costs associated with relative performance in larger fish confers a negative allometry to U_{crit}.

3.4.2.2 Effects of temperature on swimming performance

Following acclimation to different temperatures representative of their thermal range, the critical swimming performance of snapper increased with increasing temperature, before reaching a plateau, and in the case of 30 g fish, declining in response to further elevation in temperature. This thermal performance curve has been demonstrated almost universally across a range of species in response to both acute and chronic temperature exposures (Fry and Hart, 1948; Brett, 1964, 1967, 1971; Griffiths and Alderice, 1972; Brett and Glass, 1973; Taylor et al., 1996; Lee et al., 2003*c*). In the present study, the limited number of acclimation temperatures for which swimming performance was assessed limits the resolution of any performance curve and the derivation of the optimum temperature at which swimming

performance is maximal ($T_{opt\ Ucrit}$). However, the highest swimming performance for 30 g fish was observed at 18 ^{o}C, whilst 140 g fish attained a similar level of performance at both 18 and 24 ^{o}C, suggesting an optimum temperature between the two. Thermal optima are often closely associated with habitat temperature, and typically lie towards the upper end of the thermal range of a species or stock (Hammer, 1995; Lee et al., 2003*c*). The optimum temperature range for swimming performance implied by the present study is therefore consistent with that which might have been expected based on the thermal history of the stock, with temperatures in Nelson Haven fluctuating seasonally between 10 and 22 ^{o}C (Appendix One). It remains unclear which functional aspects of swimming performance are important in determining the acute and chronic thermal sensitivities of U_{crit}; although the thermal sensitivities of aerobic scope, and cardiorespiratory, nervous and muscle functions have been considered in isolation, a lack of integration between studies prevents determination of the relative importance of each under different thermal conditions (Taylor et al., 2008). Further, the thermal response of swimming performance likely differs between fish that are acutely exposed and those that have been acclimated to a given temperature, and with the direction and magnitude of temperature change.

The increase in aerobic scope at elevated temperatures facilitates a greater capacity for aerobic swimming performance; however, at temperatures above $T_{opt\ AS}$, the reduction in scope constrains performance. The thermal performance curves for aerobic scope and U_{crit} are therefore often closely matched, and by extension, $T_{opt\ AS}$ and $T_{opt\ Ucrit,}$ are often coincident (Brett, 1964, 1971; Farrell, 2002). Within the resolution afforded by the limited number of acclimation temperatures assessed during the present study, this also appears to be the case for 140 g snapper. The reason for the decline in U_{crit} in 30 g fish at 24 ^{o}C, despite scope being similar to that at 18 ^{o}C is unknown. The cost of transport is known to increase with temperature (Rao, 1968; Jones and Randall, 1978; Lee et al. 2003*b*,, 2003*c*), such that a similar aerobic scope may be less capable of supporting performance at higher temperatures.

Muscle function is also purported to be limiting to sustainable swimming performance in response to temperature change, in particular at reduced temperatures (Randall and Brauner, 1991; Taylor et al., 2008; Day and Butler, 2005). Locomotion at a given speed requires the same mechanical power output be generated by the muscle, almost irrespective of temperature; the increased drag associated with greater water viscosity at lower temperature is often considered negligible in the context of the total cost of locomotion (Rome, 1990;

Randall and Brauner, 1991). Kinematic studies indicate that sarcomere excursion, tailbeat frequency and stride length are largely independent of temperature (Rome et al., 1990; Johnston, 1993; Dickson et al., 2002; but see Sisson and Sidell, 1987) hence the velocity at which the muscle contracts must be the same for a given swimming speed. At cooler temperatures, the muscle produces less force per unit cross-section than it does contracting at the same rate under warmer conditions, possibly as a function of altered myosin ATPase function, reduced force development by individual cross-bridges, Ca^{2+} release and tropomyosin dynamics and reduced Ca^{2+} uptake by the SR and subsequent relaxation. To compensate for the reduced force (and hence power) production, additional muscle fibres are recruited, and the pattern of recruitment of motor units that occurs with increased swimming speed becomes compressed into a reduced velocity range – the so-called "compression of recruitment order" proposed by Rome and colleagues (Rome et al., 1984, 1985, 1990, 1992). As a result of this compression, the recruitment of WM at comparatively lower swimming speeds may ultimately limit swimming performance at reduced temperatures. For example, in carp acclimated to 10 and 26 ^{o}C respectively, WM recruitment was recorded by EMG at speeds of 31 and 43 cm s^{-1} respectively (Rome et al., 1984). Similarly, WM recruitment was recorded in striped bass (*Morone saxatilis*) acclimated to 9 and 25^{o}C at 1.95 and 2.82 bl s^{-1} respectively (Sisson and Sidell, 1987).

On acclimation to low temperatures, fish may demonstrate alterations in muscle function and structure to compensate for the reduced force output of fibres. The nature and the extent of these changes are species- and temperature-specific, but may include both hypertrophy and hyperplasia in the RM (Sisson and Sidell, 1987; Johnston, 1993); changes in the contractile function of the muscle through changes in the expression of myosin ATPase isoforms, and hence in ATPase activity (Johnston et al., 1975); and increased capillary and mitochondrial densities to offset the temperature-induced reduction in biological reaction rates and ensure adequate oxidative capacity to support other acclimatory changes in muscle function (Johnston and Dunn, 1987; Sidell and Moreland, 1989; Guderley, 2004). Although time constraints prevented the determination of the ultrastructural changes that occur in snapper muscle during temperature acclimation, and despite the absence of performance measures in snapper acutely exposed to different temperatures, it is evident that acclimation is partial at best, since swimming speed remains reduced in fish acclimated to cooler temperatures.

3.4.3 Recovery metabolism and EPOC

Following the cessation of swimming activity, and hence the decline in metabolic demand by contractile muscle elements, $\dot{M}O_2$ remains elevated. This "excess post-exercise oxygen consumption (EPOC)" represents the aerobic cost associated with the correction of exercise-induced perturbations, including the replenishment of ATP and PCr, oxidation of lactate for subsequent glycogen restitution, the recharging of intracellular O_2 stores, and the correction of ionic and osmotic imbalance (Scarabello et al., 1991, 1992; Wood, 1991). Post-exercise $\dot{M}O_2$ may therefore be considered a measure of recovery, since presumably the return of $\dot{M}O_2$ to routine levels is indicative of the correction of exercise-related disturbance and reinstatement of a rested condition (Lee et al., 2003*b*).

Studies of EPOC in fish are surprisingly few. Brett (1964) determined that the recovery of $\dot{M}O_2$ in sockeye salmon exercised to U_{crit} required approximately 3 hours, while several studies of the recovery of juvenile rainbow trout chased to exhaustion have suggested recovery times of 3 to 6 hours (Scarabello et al., 1991, 1992). In contrast, $\dot{M}O_2$ is reported to return to pre-exercise rates in as little as 1.5 hours in Atlantic cod (Reidy et al. 1995), and between 20 to 80 minutes in different stocks of sockeye salmon (Lee et al., 2003*a*,2003*b*). During the present study, the time required for $\dot{M}O_{2\ \text{post-exercise}}$ to return to pre-exercise rates varied between 1.5 and >24 hours, depending on fish size and acclimation temperature. However, in some groups – notably 30 g fish acclimated to 18 and 24 ^{o}C – fish appeared to establish a post-exercise $\dot{M}O_{2\ \text{routine}}$ lower than that exhibited pre-exercise, hence recovery time and EPOC are likely to be underestimated in these groups. Subtle variation in pre- and post-exercise $\dot{M}O_2$ has been documented previously for other species, likely resulting from changes in spontaneous activity post-exercise (Reidy et al., 1995; Fu et al., 2009). In these instances, it is possibly more accurate to utilise estimates of post-exercise $\dot{M}O_{2\ \text{routine}}$ for the determination of EPOC and recovery time, since they will account for any changes in spontaneous energy expenditure. Post-exercise $\dot{M}O_{2\ \text{routine}}$ was therefore determined as the mean $\dot{M}O_2$ between 18 and 24 hours post-exercise, and EPOC and recovery time recalculated as before. The results are presented in Table 3.4, and suggest more accurate estimates of recovery time may be between 5 and 18 hours, again depending on size and temperature. Interestingly, despite the effects of both temperature and mass on metabolic rate being well-documented, we are aware of no systematic study in which the possible relation between either temperature or size and $\dot{M}O_{2\ \text{post-exercise}}$ or EPOC has been explored, although several

Table 3.4. Estimates of excess post-exercise oxygen consumption (EPOC) and recovery time in snapper, using estimates of post-exercise $\dot{M}O_{2\ routine}$.

Mass (g)	Temperature (°C)	Post-exercise $\dot{M}O_{2\ routine}$ (μmol g^{-1} h^{-1})	EPOC (μmol g^{-1})	Recovery time (h)
30	12	2.81 ± 0.17	10.1	11.0
	18	4.74 ± 0.21	24.2	8.0
	24	8.05 + 0.46	25.6	5.0
140	12	1.72 ± 0.06	17.3	17.5
	18	3.25 ± 0.30	28.1	18.5
	24	7.15 ± 0.57	32.0	8.0

studies in which their effects on the disturbance and recovery of metabolite status are determined are discussed below.

3.4.3.1 Effects of size on recovery from fatigue

In the present study, larger snapper appeared to experience a greater EPOC and a prolonged recovery time, when compared with smaller fish at the same temperature. The magnitude of exercise-induced perturbations has previously been implied to vary with size; rainbow and brook trout (*Salvelinus fontinalis*) exercised to exhaustion exhibited a 2-fold increase in WM lactate accumulation, with parallel changes in pH_i, as body length increased from 8 to 39 cm and 8 to 54 cm, respectively (Ferguson et al., 1993; Kieffer et al., 1996). Although neither study investigated the possible differences in subsequent recovery that may occur with size, the greater metabolic disturbance experienced by larger fish is presumably associated with an increased cost (i.e. EPOC) and duration of recovery (Kieffer, 2000). The relationship between size and recovery from exercise may, however, be species-specific; the accumulation of metabolic end products in the WM of the largemouth bass, a sit and wait predator, was independent of size (10 to 36 cm; Kieffer et al., 1996). Hence, the lifestyle of a species may be important in determining the biological constraints on performance. That snapper should exhibit a size-related difference in EPOC and recovery time would be

consistent with the inference of differential metabolic disturbance with size in more athletic fish species.

3.4.3.2 Effects of temperature on recovery from fatigue

Temperature also had an influence on the recovery from exhaustive exercise, with higher EPOC and a more rapid recovery observed at warmer acclimation temperatures. The few existing studies of the effects of temperature on the magnitude of exercise-induced metabolic disturbance have yielded equivocal results; while some studies have reported the temperature-independence of metabolic disturbance (Wilkie et al., 1997; Hanna et al., 2008*b*), others have found that, to varying degrees, increasing temperature exacerbates the metabolic perturbations associated with exercise, resulting in greater depletion of PCr, ATP, and glycogen, and a greater accumulation of lactate (Dalla Via et al., 1989; Kieffer et al., 1994; Sfakianakis and Kentouri, 2010). Elevated plasma lactate levels have also been documented at higher temperatures; however, as the release of lactate to the plasma is known to be temperature-dependent, such measurements may not reliably indicate the effects of temperature on WM metabolic load (Jain and Farrell, 2003). Presumably, these greater disturbances are associated with a higher cost of correction (i.e. EPOC) that would be consistent with the higher EPOC observed in larger snapper.

The effects of temperature on the metabolic recovery from exercise appear more consistent; with the exception of PCr, the recovery of metabolic disturbance (that is, the restoration of ATP and glycogen, and clearance of lactate) is expedited at warmer temperatures (Kieffer et al., 1994; Wilkie et al., 1997; Galloway and Kieffer, 2003; Sfakianakis and Kentouri, 2010). The increase in recovery rate associated with elevated temperature likely results from changes in diffusive and enzymatic reaction rates, accelerating the rates of reactions involved in restorative processes (Kieffer et al., 1994; Wilkie et al., 1997; Kieffer, 2000). Similarly, Brett (1964) found that despite greater EPOC at higher temperatures, suggestive of greater metabolic disturbance, recovery time was independent of temperature, presumably because of the acceleration of corrective processes. The more rapid recovery in snapper acclimated to increasingly warmer temperatures would be consistent with the studies discussed above.

3.5 Conclusions

The present study sought to investigate the metabolic and swimming capacities of juvenile snapper, and the potential influence of changes in size and temperature. Metabolic rates demonstrated classic allometric and temperature relations. Smaller snapper exhibited higher rates of mass-specific oxygen consumption than did larger fish under similar conditions, with mass exponents of 0.74 and 0.81 determined for $\dot{M}O_{2\ routine}$ and $\dot{M}O_{2\ max}$, respectively. No size-dependence in absolute aerobic metabolic scope was evident over the size rate examined, hence the potential for size to impact aerobic performance will depend on the nature of the activity and the scaling of any inherent metabolic costs. Both $\dot{M}O_{2\ routine}$ and $\dot{M}O_{2\ max}$ increased with increasing ambient temperature, with $T_{opt\ AS}$ estimated to be approximately 24 ^{o}C, consistent with what might have been expected based on the thermal history of the snapper. Presumably, aerobic scope becomes constrained with further elevation in temperature.

These are the first reported measurements of swimming capacity in snapper. Fish exhibited a strong swimming capacity, with swimming speeds of up to 7.1 bl s^{-1} observed. Swimming performance was correlated with size, with critical swimming speed (in absolute terms) proportional to $(length)^{0.62}$, such that while larger fish sustained higher absolute velocities, smaller fish were capable of higher relative performance. Swimming performance was also sensitive to changes in temperature; the thermal sensitivity of U_{crit} for 140 g snapper appeared to mirror that of aerobic scope in those fish, however smaller snapper experienced a decline in performance at temperatures where $T_{opt\ AS}$ was implied.

Following exhaustion at U_{crit}, the period required for $\dot{M}O_2$ to return to stable, routine rates varied between approximately 5 and 18.5 hours, depending on the size of the fish and its acclimation temperature. Larger snapper experienced a higher EPOC, indicative of a greater anaerobic disturbance within the WM, and which was associated with a prolonged recovery period. Fish acclimated to warmer temperatures similarly experienced a higher EPOC, however recovery was expedited, presumably a function of the increased rate of diffusive and catalytic reactions accelerating the restoration of homeostasis.

CHAPTER 4

The repeat swimming performance of juvenile snapper

4.1 Introduction

Swimming performance is considered to be one of the main determinants of fitness and survival in fish, with relevance to foraging success, predator avoidance, reproductive activities, migration and dispersal (Videler, 1993; Kieffer, 2000; Plaut, 2001; Hanna et al., 2008*a*; Oufiero and Garland, 2009), as well as anthropogenic interactions, such as the negotiation of fish passes (Jones et al., 1974; Peake, 2008) or avoidance of fishing gears (He, 1993; Winger et al., 2010). Critical swimming speed, U_{crit}, is a commonly used yet complex measure of swimming capacity, incorporating both aerobic and anaerobic capacities; low velocity swimming is fuelled by aerobic metabolism in the red muscle (RM), with anaerobically-fuelled white muscle (WM) increasingly recruited at velocities approaching U_{crit}. While sustained locomotion results in minimal physiological disturbance (Wilson and Egginton, 1994; Richards et al., 2002*b*), high intensity, burst-type activity induces severe metabolic, acid-base, ionic, osmotic and hormonal perturbations, and ultimately ends in fatigue (see reviews by Wood and Perry, 1985; Wood, 1991; Milligan, 1996). The correction of these perturbations, in particular the clearance of accumulated metabolites and replenishment of energy reserves, are likely important in determining when subsequent high-intensity activity may occur (Suski et al., 2007; Kieffer et al., 2011).

The recovery of swimming performance following an exhaustive event is an important aspect of exercise capacity, with significant ecological implications; for example, impaired swimming performance has the potential to compromise behaviours such as predator avoidance or foraging (Jain et al., 1998; Ryer, 2004; Lee-Jenkins et al., 2007; Kieffer et al., 2011). Yet, in comparison with the extensive literature that exists detailing the swimming performance of an array of species under various environmental conditions (reviewed by

Beamish, 1978; Hammer, 1995; Kieffer, 2000), and of the magnitude and recovery of exercise-induced metabolic perturbations (Wood and Perry, 1985; Wood, 1991; Milligan, 1996), information detailing how quickly or how completely swimming capacity recovers following exhaustive exercise is limited. For the most part, studies of repeat swimming performance have focused on salmonids, since the potential for these fish to encounter multiple, high-intensity exercise events during their upstream migration is high, and with direct implications for their ability to reach spawning grounds (Jain et al., 1998; Farrell et al., 2003; MacNutt et al., 2004). These studies demonstrate that salmonids are able to perform equally well in repeat U_{crit} tests, where the recovery period between swimming challenges is as little as 40 minutes (Table 4.1). The recovery of critical swimming capacity may, however, be influenced by environmental conditions, including temperature (Jain and Farrell, 2003), hypoxia (Farrell et al., 1998) and the presence of toxicants (Jain et al., 1998), and biotic factors such as species (Farrell et al., 2003), physical condition (Jain et al., 1998) and whether animals are sourced from wild or hatchery-raised stocks (Brauner et al., 1994; Lee et al., 2003*a*). The rapid recovery of swimming performance following an exhaustive event suggests that full metabolic recovery, which may take 6-24 hours (Wardle, 1978; Milligan and Wood, 1986; Richards, 2002*a*), is not required for maximal swimming activity to occur. Surprisingly, there appears to have been no attempt to discern the duration of any impairment in critical swimming performance; previous studies have utilised a single recovery period, after which they have concluded whether or not swimming capacity has been restored. The exception is preliminary experimentation by Jain et al. (1998), where rainbow trout exhibited equivalent U_{crit} performance following recovery periods of 70 to 120 minutes, with recovery ratios declining to 0.79 and 0.92 in two fish provided a 60 minute recovery period.

The recovery of swimming capacity following an exhaustive event is likely an important consideration to fisheries management, not only with respect to understanding the migration of anadromous fish, but also in attempting to understand the fate of fish that interact with, and subsequently escape or are discarded from, fishing gears. In trawl-based fisheries, the most common means of attempting to reduce the bycatch of non-target and juvenile fish are restrictions of net mesh size, changes in mesh geometry and/or the inclusion of sorting grids, to allow undersized fish to escape at depth (Chopin and Arimoto, 1995; Suurnonen, 2005). However, such measures do not prevent fish from interacting with the fishing gears, and fish remain exposed to the stresses associated with the capture process, including forced exercise,

Table 4.1. Experimental recovery periods, after which repeat swimming performance was equal to that of the initial swimming challenge (i.e., $U_{crit\ 1} = U_{crit\ 2}$), in several species of salmonid.

Species	Recovery period	Recovery velocity	Reference
Sockeye salmon	40 min	0.3 bl s^{-1}	Jain et al., 1998
	45 min	0.4 bl s^{-1}	Farrell et al., 1998
	45 min	0.45 bl s^{-1}	Farrell et al., 2003
	45 min	0.3-0.45 bl s^{-1}	Lee et al., 2003*b*
	45 min	0.3-0.45 bl s^{-1}	Lee et al., 2003*c*
Coho salmon	45 min	0.45 bl s^{-1}	Farrell et al., 2003
	45 min	-	Lee et al., 2003*a*
	45 min	0.3-0.45 bl s^{-1}	Lee et al., 2003*b*
	45 min	0.3-0.45 bl s^{-1}	Lee et al., 2003*c*
	120 min	1.3 bl s^{-1}	Brauner et al., 1994
Rainbow trout	40 min	0.25 bl s^{-1}	Jain and Farrell, 2003
	90 min	0.3 bl s^{-1}	Jain et al., 1998
Chinook salmon	60 min	2 bl s^{-1}	Randall et al., 1987
Cutthroat trout	45 min	0.45 bl s^{-1}	MacNutt et al., 2004
Pink salmon	45 min	0.45 bl s^{-1}	Farrell et al., 2003

- denotes no mention of recovery velocity within the cited reference

Species names: Sockeye salmon, *Oncorhynchus nerka*; coho salmon, *Oncorhynchus kisutch*; rainbow trout, *Oncorhynchus mykiss*; Chinook salmon, *Oncorhynchus tshawytcha*; cutthroat trout, *Oncorhynchus clarki clarki*; pink salmon, *Oncorhynchus gorbuscha.*

confinement, and impact and crush injuries (Chopin and Arimoto, 1995; Davis, 2002; Ryer, 2004; Suuronen, 2005; Broadhurst et al., 2006). Field studies and laboratory-based simulations of commercial fishing practices indicate that mortality in escaping and discarded fish may be extensive (up to 100%; Chopin and Arimoto, 1995; Davis, 2002; Suuronen, 2005; Broadhurst et al., 2006), while fish that experience sub-lethal stresses may suffer indirect mortality resulting from physiological or behavioural deficits. For example, juvenile walleye pollock (*Theragra chalcogramma*; Ryer, 2002) and sablefish (*Anoplopoma fimbria*; Ryer et al., 2004) subjected to simulated trawl capture exhibited reduced routine swimming speeds and shoal cohesion, and altered predator evasion behaviours, and were consequently more vulnerable to predation. The critical swimming performance of brook trout (*Salvelinus fontinalis*) was reduced by up to 75% following simulated angling stress and air exposure (Schreer et al., 2005). The recovery of swimming performance following a metabolically

taxing event may therefore be fundamental to correcting behavioural impairments that may compromise fitness or survival.

4.1.1 Objectives

The aim of the present study was to investigate the repeat swimming performance of juvenile snapper (*Pagrus auratus*) following an exhaustive event, including the degree and duration of any impairment in critical swimming speed, through the use of several different recovery periods. Where experimental design permitted, metabolic rate was also determined following initial and repeat swimming challenges, to provide an indication of metabolic status.

4.2 Materials and Methods

4.2.1 Repeat swimming performance

Critical swimming speed was determined in accordance with the U_{crit} incremental velocity test described in Section 2.3. Briefly, fish were placed into the central tunnel of a Blazka-type swimming flume for a 30 minute habituation period, during which the water velocity was set to 0.5 bl s^{-1}. The exercise test commenced with the increase of water velocity to 1 bl s^{-1}, with velocity increased in 1 bl s^{-1} increments at 15 minute intervals thereafter, until the fish was exhausted and became impinged on the rear mesh of the flume. The water flow was stopped and the time and speed of exhaustion recorded. U_{crit} determinations were performed individually, and at a temperature of 18 ^{o}C.

Following an initial exhaustive U_{crit} test ($U_{crit\ 1}$), a second test ($U_{crit\ 2}$) was performed, separated by a recovery period of 0, 1 or 2 hours (30-75 g; each group, n=8). For fish immediately subject to $U_{crit\ 2}$ (i.e. 0 h), water velocity was reset to 1 bl s^{-1}, and the U_{crit} protocol repeated. Fish provided a 1 or 2 hour recovery period were removed from the flume following exhaustion, and translocated to respirometers, as described in Section 2.4 (and see Section 4.2.2 below), for the intervening period, to allow the determination of $MO_{2\ post\text{-}exercise}$.

No habituation period was observed prior to $U_{crit\ 2}$; on return to the flume, water velocity was set to 1 bl s^{-1} and subsequently increased as per the initial test.

Given the rapid recovery of swimming performance following a recovery period of 0 to 2 hours (see Section 4.3.1), and the demonstration by previous studies (Milligan, 2000; Farrell et al., 2001) that low to moderate intensity exercise may expedite metabolic recovery, a further set of experiments was employed to investigate the degree and duration of impairment of critical swimming speed. Following the determination of $U_{crit\ 1}$, fish (85-160g) were subject to an "abridged" version of the U_{crit} test. Immediately following exhaustion, the flow of water within the flume was stopped to allow the fish to move off of the rear mesh; water velocity was then rapidly increased (over approximately 10 seconds) to either 50, 60 or 75% of the initial U_{crit} velocity (each, n = 8). Thereafter, water velocity was increased in 1 bl s^{-1} increments at 15 minute intervals as per the original protocol, until the fish again became impinged on the rear mesh of the flume. The water current was stopped and the time and speed at fatigue recorded.

4.2.2 $MO_{2\ post\text{-}exercise}$

The provision of a 1 or 2 hour recovery period allowed for the recording of oxygen consumption following both $U_{crit\ 1}$ and $U_{crit\ 2}$. In these fish, routine metabolic rate was first established by placing the fish within the respirometers 18 hours prior to the determination of oxygen consumption, to allow recovery from handling-related stresses. $MO_{2\ routine}$ was then determined as described in Section 2.4, by calculating the rate of oxygen depletion over a minimum 1 hour period. PO_2 within the respirometer was maintained between 120 and 140 mmHg, ensuring normoxic conditions. Following the measurement of $MO_{2\ routine}$, fish were transferred to the swimming flume for the determination of $U_{crit\ 1}$, and following exhaustion, were promptly transferred back to the respirometer for the allotted recovery period. After this time, the fish were replaced into the flume for measurement of $U_{crit\ 2}$, after which they were again transferred to the respirometers for the measurement of $MO_{2\ post\text{-}exercise}$. $MO_{2\ post\text{-}exercise}$ was calculated at 15 minute intervals for the period following $U_{crit\ 1}$, and at 15 minute intervals for the first two hours, and 30 minute intervals for the subsequent eight hours following $U_{crit\ 2}$. $MO_{2\ max\ 1}$ and $MO_{2\ max\ 2}$ describe the maximum rates of oxygen consumption during each of these respective periods.

4.2.3 Statistical analysis

Differences in the mass, length and condition factor of fish between recovery groups were each analysed using one-way ANOVA; where a significant result was indicated, a Tukey Multiple Comparisons test was employed to discern which groups were different from each other.

$U_{crit\ 1}$ and $U_{crit\ 2}$ were compared for individual fish within each recovery group using a Student's t-test of paired design. The recovery ratio (*R*), the quotient of $U_{crit\ 2}$ / $U_{crit\ 1}$, was calculated for each fish. As body size was significantly defferent between recovery groups, differences in $U_{crit\ 1}$, $U_{crit\ 2}$ and *R* between groups were subsequently compared using ANCOVA, with fish length as a covariate. Tukey Multiple Comparisons of the adjusted means were used to identify specific differences between recovery groups.

For individual fish provided a 1 or 2 hour recovery period, the following comparisons were made using a paired Student's t-test: $MO_{2\ routine}$ and $MO_{2\ max\ 1}$; $MO_{2\ routine}$ and $MO_{2\ max\ 2}$; $MO_{2\ max\ 1}$ and $MO_{2\ max\ 2}$. Comparisons of $MO_{2\ routine}$ and $MO_{2\ max}$ between fish in the 1 and 2 hour recovery groups were performed using a Student's t-test of unpaired design.

The recovery of $MO_{2\ post\text{-}exercise}$ following both $U_{crit\ 1}$ and $U_{crit\ 2}$ was analysed by the pair-wise comparison of $MO_{2\ routine}$ with $MO_{2\ post\text{-}exercise}$ at subsequent sampling times, using a two-tailed Student's t-test of paired design (Scarabello et al., 1991, 1992). Excess post-exercise oxygen consumption (EPOC) following $U_{crit\ 2}$ was determined by calculating the area bound by the $MO_{2\ post\text{-}exercise}$ curve and a y-value equal to $MO_{2\ routine}$, between the time of exhaustion and the time at which $MO_{2\ post\text{-}exercise}$ is equal to $MO_{2\ routine}$ (Lee et al., 2003*a*, 2003*b*).

A key to studying traits at the individual levels is to understand the amount of intraindividual variation, or repeatability, of the trait (Oufiero and Garland, 2009) – in this case U_{crit}. The experimental repeatability of U_{crit} was assessed using Spearman's rank correlation (Gregory and Wood, 1998; Kolok, 1999); only data from the 1 and 2 hour recovery groups, where mean recovery ratios were ≥ 0.95 were used for this analysis.

In all cases, significance was taken at the level of $p \leq 0.05$.

4.3 Results

As a result of repeat swimming performance being assessed in two distinct phases (0, 1, and 2 hour recovery groups in March 2012; 50, 60 and 70% $U_{crit\ 1}$ groups in May 2013), differences in size were evident between groups (Table 4.2), notably that those fish where the abridged $U_{crit\ 2}$ test was used were larger and had a higher condition factor than those used in the initial set of recovery determinations.

Critical swimming performance within individuals was experimentally repeatable (Spearman's rank correlation coefficient, $\rho = 0.8449$, $p < 0.0001$). No fish refused to swim outright during repeat swimming challenges.

4.3.1 Repeat swimming performance

Critical swimming speed ranged from 4.27 to 6.07 bl s^{-1}, and having accounted for the variation in fish size between recovery groups, there was no significant variation in $U_{crit\ 1}$ between groups ($p = 0.3372$). Repeat swimming performance was dependent on the recovery period between swimming events ($p < 0.0001$): $U_{crit\ 2}$ was significantly reduced when immediately preceded by $U_{crit\ 1}$, but was progressively restored following longer recovery periods, such that snapper were able to repeat their initial $U_{crit\ 1}$ performance following a 2 hour recovery period (Fig. 4.1, 4.2).

During the abridged tests in which $U_{crit\ 2}$ commenced at a velocity 50 or 60% of $U_{crit\ 1}$, snapper were able to attain an average critical swimming speed of 3.69 and 3.76 bl s^{-1} respectively – some 72% of their initial swimming capacity (Fig 4.1, Fig. 4.2). When the water velocity during the successive U_{crit} test was set to 75% of the velocity of $U_{crit\ 1}$, three fish recorded a $U_{crit\ 2}$ between 3.69 and 4.12 bl s^{-1}. The remaining fish in this group failed to swim following 1 to 5 minutes (mean 2.5 $\pm$ 0.8) at 75% of $U_{crit\ 1}$. In the absence of a penultimate sustainable speed, $U_{crit\ 2}$ could not be calculated for these fish, and hence $U_{crit\ 2}$ and *R* are not displayed in Figures 4.1 and 4.2.

4.3.2 Post-exercise oxygen consumption

Oxygen consumption was determined for fish in the 1 and 2 hour recovery groups, where the period between swimming trials allowed the determination of $\dot{M}O_{2\ max\ 1}$, for subsequent comparison with $\dot{M}O_{2\ max\ 2}$ following the repeat swimming test. Following overnight habituation in the respirometers, $\dot{M}O_{2\ routine}$ was similar between 1 and 2 hour recovery group fish, at 4.95 $\pm$ 0.51 and 5.66 $\pm$ 0.34 µmol g^{-1} h^{-1}, respectively. $\dot{M}O_2$ was elevated between 2.0 and 2.3-fold above routine levels following both $U_{crit\ 1}$ and $U_{crit\ 2}$ in both recovery groups (Table 4.1, Fig. 4.3; all $p < 0.0010$). $\dot{M}O_{2\ max\ 1}$ was significantly higher in fish in the 2 hour recovery group than the 1 hour group ($p = 0.0015$); this appeared to result in a more rapid recovery of $\dot{M}O_{2\ post\text{-}exercise}$ towards routine level in the 1 hour group, although $\dot{M}O_2$ did not reach routine levels in either group prior to their second U_{crit} challenge. Following $U_{crit\ 2}$, $\dot{M}O_{2\ max\ 2}$ was similarly elevated: $\dot{M}O_{2\ max\ 2}$ was similar to $\dot{M}O_{2\ max\ 1}$ in the 2 hour recovery group ($p = 0.3811$), while $\dot{M}O_{2\ max\ 2}$ was significantly higher than $\dot{M}O_{2\ max\ 1}$ for 1 hour fish ($p = 0.0208$), and was instead similar to $\dot{M}O_{2\ max\ 1}$ and $\dot{M}O_{2\ max\ 2}$ of the 2 hour recovery group ($p = 0.5041$).

The time taken for $\dot{M}O_2$ to return to routine levels following $U_{crit\ 2}$ was 1.5 and 3.5 hours in 1 hour and 2 hour recovery group fish, respectively. The EPOC associated with recovery from $U_{crit\ 2}$ was estimated as being 7.1 and 7.0 µmol g^{-1} for 1 and 2 hour recovery groups, respectively.

Table 4.2. Repeat swimming performance and oxygen consumption in juvenile snapper following various recovery periods.

Recovery group	Mass (g)	Length (mm)	CF	U_{crit} (bl s^{-1})		Recovery ratio, *R*	$\dot{M}O_2$ (µmol g^{-1} h^{-1})		
				$U_{crit\ 1}$	$U_{crit\ 2}$		$\dot{M}O_{2\ rest}$	$\dot{M}O_{2\ max\ 1}$	$\dot{M}O_{2\ max\ 2}$
75% $U_{crit\ 1}$	127.2 ± 6.2[a]	173 ± 2 [a]	2.43 ± 0.05 [a]	5.19 ± 0.09 [a]					
60% $U_{crit\ 1}$	116.8 ± 4.3 [a]	171 ± 2 [a]	2.35 ± 0.04 [ab]	5.15 ± 0.16 [a]	3.69 ± 0.15 [a]	0.72 ± 0.03 [a]			
50% $U_{crit\ 1}$	117.3 ± 5.2 [a]	169 ± 2 [a]	2.42 ± 0.06 [a]	5.27 ± 0.09 [a]	3.76 ± 0.04 [a]	0.72 ± 0.01 [a]			
0 h	60.9 ± 4.4 [b]	140 ± 3 [b]	2.20 ± 0.05 [bc]	4.80 ± 0.16 [a]	4.17 ± 0.13 [ab]	0.87 ± 0.03 [b]			
1h	54.2 ± 3.0 [b]	138 ± 3 [b]	2.04 ± 0.05 [cd]	5.06 ± 0.17 [a]	4.81 ± 0.2 [bc]	0.95 ± 0.02 [bc]	4.95 ± 0.51 [a]	9.75 ± 0.72 [a]	11.37 ± 1.04 [a]
2h	46.4 ± 3.0 [b]	133 ± 3 [b]	1.97 ± 0.03 [d]	4.97 ± 0.24 [a]	4.85 ± 0.24 [c]	0.98 ± 0.02 [c]	5.66 ± 0.39 [b]	12.81 ± 0.74 [a]	12.25 ± 0.76 [a]

Letters denote significant differences ($p < 0.05$) between recovery groups

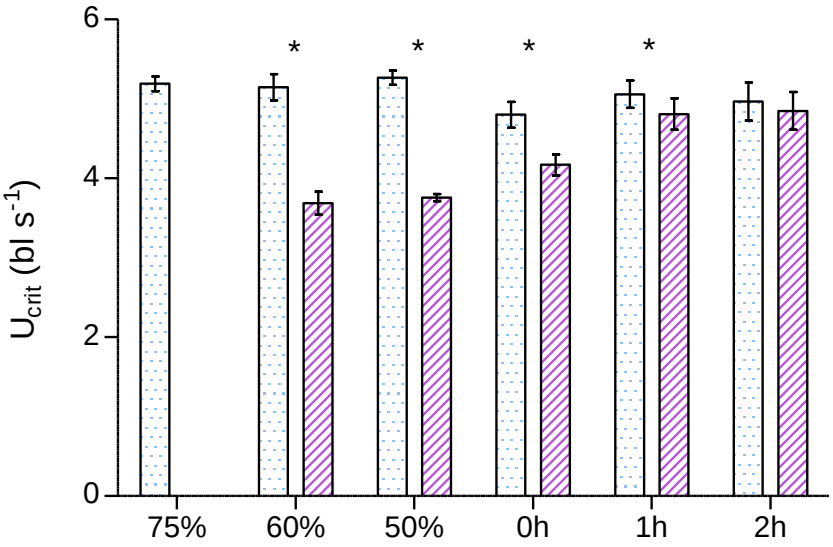


Figure 4.1. Critical swimming speed of snapper during initial ($U_{crit\ 1}$, ▭) and repeat ($U_{crit\ 2}$, ▨) exercise tests. Groups refer to fish provided a recovery period of 0, 1 or 2 hours prior to the repeat test, or provided an abridged version of $U_{crit\ 2}$, whereby the test commenced at a water velocity of 50, 60 or 75% of the speed of $U_{crit\ 1}$. $U_{crit\ 2}$ data are not presented for the 75% group as this could not be calculated for all fish within the group. * indicates a significant difference between $U_{crit\ 1}$ and $U_{crit\ 2}$ within a recovery group.

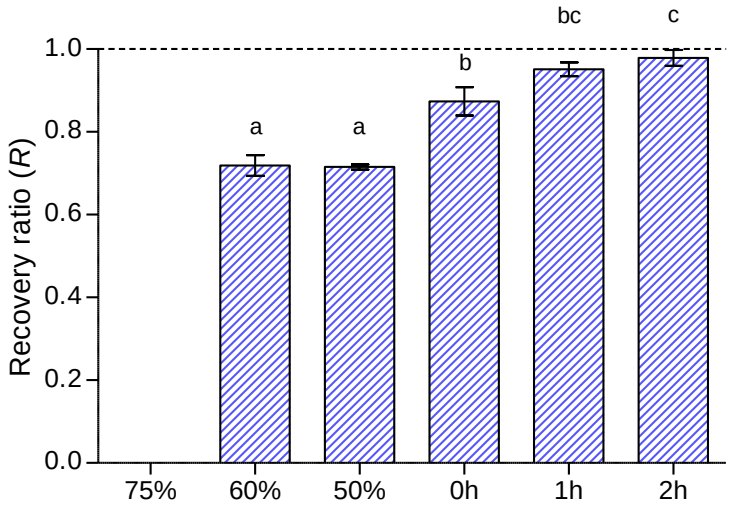


Figure 4.2. Recovery ratio, *R*, of swimming performance ($U_{crit\ 2}$ / $U_{crit\ 1}$) following different recovery periods. The dashed line identifies a recovery ratio of unity. Letters denote significant differences in *R* between recovery groups.

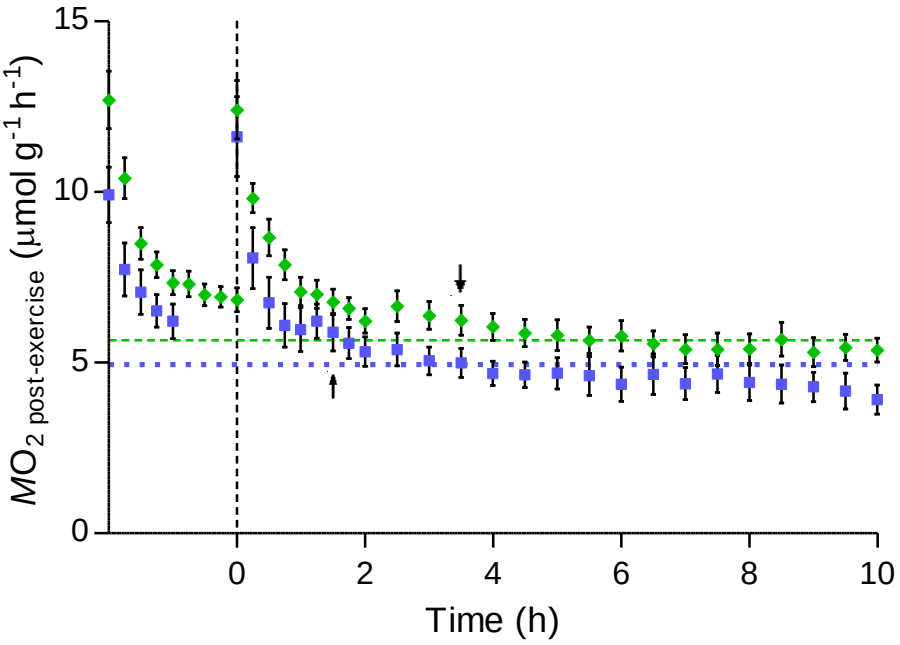


Figure 4.3. Oxygen consumption (MO_2) following $U_{crit\ 1}$ and $U_{crit\ 2}$ for 1 hour (■) and 2 hour (◆) recovery group fish. The dashed vertical line indicates the second swimming event, $U_{crit\ 2}$, with the 2 hour block prior to this showing MO_2 during the 1 or 2-hour recovery period between swimming events. The dotted horizontal line represents the mean $MO_{2\ routine}$ for the 1 hour recovery group, and the dashed horizontal line the mean $MO_{2\ routine}$ for the 2 hour recovery group. For clarity, rather than indicate all of the post-exercise values which are significantly different to $MO_{2\ routine}$, the point at which pre- and post-exercise metabolic rates are no longer significantly different are indicated with an arrow.

4.4 Discussion

Swimming performance is a key determinant of fitness and survival in fish, with the ability to sprint or sustain high swimming speeds crucial to numerous routine activities, including foraging and predator avoidance (Kieffer, 2000; Plaut, 2001; Hanna et al., 2008*a*; Oufiero and Garland, 2009). High intensity exercise often results in fatigue, characterised by an inability to perform further burst activity (Wood, 1991; Milligan, 1996). While recovery from exercise is often measured with reference to metabolite concentrations, the recovery of swimming capacity itself is more likely of direct ecological relevance, ultimately determining the ability of an animal to perform subsequent behaviours, such as predator avoidance or location of a refuge in which to recover completely (Jain et al., 1998; Kieffer et al., 2011). The present study represents one of the first to examine in detail the degree, and in particular, the duration of impairment of critical swimming performance following an exhaustive event.

4.4.1 The repeat swimming performance of snapper

The critical swimming performance of snapper was reduced following an exhaustive exercise challenge, but was rapidly restored thereafter; snapper were able to attain 87% of their initial U_{crit} performance when challenged with a second U_{crit} test immediately following the termination of the first. Repeat swimming performance increased to 95 and 98% of $U_{crit\ 1}$ when tests were separated by intervals of 1 and 2 hours, respectively. This rapid recovery of critical swimming performance is consistent with that seen in salmonids (Table 4.1), where fish are able to perform equally well in U_{crit} tests separated by as little as 40 minutes. U_{crit} values obtained for the initial swimming performance of snapper are consistent with those predicted by the allometric equations derived in Chapter 3 of this thesis.

The ability of fish to sustain swimming at low to moderate velocities immediately following exhaustion has been well documented (Table 4.1; Milligan et al., 2000; Farrell et al., 2001*a*, 2001*b*; Kieffer et al., 2011). For example, rainbow trout were capable of swimming at speeds of 80% U_{crit} for 4 hours (Primmett et al., 1986) and Chinook salmon at 2 bl s^{-1} (63-80% $U_{crit\ 1}$) for 2 hours (Randall et al., 1987) following fatigue during respective exhaustive exercise challenges. High intensity exercise results in only minimal disturbance within the red muscle (RM); rainbow trout swimming for 45 minutes at 90% U_{crit} exhibited a

~50% reduction in RM PCr and glycogen, and only low levels of lactate accumulation or lipid depletion (Richard et al., 2002*b*). Despite the acidification of the blood that results during burst activity, oxygen delivery to the tissues is maintained through the action of catecholamines, which preserve the contractile function of cardiac tissue (Farrell, 1985), and stimulate the regulation of erythrocyte pH_i, preventing Root and Bohr shifts in the haemoglobin that could significantly compromise oxygen-loading at the gills (Primmett et al., 1986; Wells and Dunphy, 2009). The RM therefore continues to function following fatigue, permitting sustainable levels of activity. In contrast, the significant perturbations that occur within the white muscle (WM) – in particular, the accumulation of H^+ and P_i – have been associated with impaired muscle function (Altringham and Johnston, 1985; Mutungi and Johnston, 1988; Westerblad, 2002; Vandenboom, 2004). Although few authors have sought to link the post-exercise recovery of metabolites with swimming performance, comparison of the existing literature indicates that complete metabolic recovery is not required for subsequent burst activity to occur; while the resynthesis of WM PCr and ATP may take as little as 15 minutes and 1 hour respectively (Scarabello et al., 1992; Wang et al., 1994*a*), the clearance of lactate and replenishment of muscle glycogen, may take 6-24 hours (Wardle, 1978; Milligan and Wood, 1986; Pagnotta and Milligan, 1991; Scarabello et al., 1991; Schulte et al., 1992; Richards et al., 2002*a*; van Ginneken et al., 2008). Indeed, fish may demonstrate the reinstatement of swimming capacity despite a residual metabolic load; sockeye salmon are capable of equivalent repeat swimming performance despite $\dot{M}O_2$ and plasma lactate being elevated and residual ionic disturbances evident prior to the start of a second swimming challenge (Farrell et al., 1998; Jain et al., 1998; Jain and Farrell, 2003). In the present study, snapper were also able to demonstrate recovery of critical swimming speed, despite $\dot{M}O_{2\ \text{post-exercise}}$ remaining elevated above routine levels. Although no attempt was made to measure changes in metabolite concentrations, a separate study of metabolic recovery following fatigue at U_{crit} indicates that for snapper, the correction of metabolic disturbance requires approximately 6 hours (see Chapter 5).

Short of requiring full metabolic recovery, there is evidence to suggest that the perturbations associated with exhaustive exercise must exceed a threshold level before adversely affecting subsequent swimming performance. Rainbow and cutthroat trout acclimated to warmer temperatures experienced a larger exercise-induced metabolic disturbance than did fish acclimated to cooler temperatures, which may have contributed to the impairment of repeat swimming performance of warm- but not cold-acclimated fish (Jain

and Farrell, 2003; MacNutt et al., 2004). Further, in warm-acclimated rainbow trout, plasma lactate concentrations in excess of 12 mmol l^{-1} were inversely correlated with $U_{crit\ 2}$ performance (Jain and Farrell, 2003). Sockeye salmon exposed to hypoxia during $U_{crit\ 1}$ developed a larger anaerobic load than did normoxic fish, with plasma lactate concentrations of 11 mmol l^{-1} and <5 mmol l^{-1} respectively prior to the start of a second (normoxic) U_{crit} test; 4 of the 5 hypoxic fish refused to swim during the repeat test, even under normoxic conditions, while control fish recorded recovery ratios of 0.97 (Farrell et al., 1998). Finally, repeat swimming performance was reduced in sockeye salmon exposed to dehydroabietic acid (DHA); although plasma lactate remained elevated in both the control and toxicant-exposed fish at the start of $U_{crit\ 2}$, lactate concentrations were higher and repeat swimming performance lower in the latter (Jain et al., 1998).

4.4.2 Post-exercise activity may expedite recovery

The apparent discrepancy between the recovery of metabolites and swimming performance may also be explained in part by the design of the U_{crit} test. Firstly, test durations are long, and may vary between studies through the differential use of velocity increments and time intervals; for example, the duration of the U_{crit} tests cited in Table 4.1 ranged between approximately 60 and 180 minutes, with most tending towards 120 minutes. In the present study, a full U_{crit} test took approximately 75 minutes. If WM function is presumed to be underlying the impairment of critical swimming performance, the term "recovery period" to describe the interval between the two swimming events is possibly misleading, as the time between the termination of the first test and WM recruitment during the second may be more relevant as a measure of recovery time. However, since WM recruitment is difficult to identify in the absence of EMG recordings or detailed kinematic analysis, the use of the term "recovery period" to describe the period between U_{crit} tests has been retained for this discussion.

Perhaps more importantly, there is evidence to suggest that the recovery of exercise-induced perturbations is expedited in fish swimming at low to moderate intensities during recovery. Milligan et al. (2000) observed that in rainbow trout chased to exhaustion, fish forced to swim at 0.9 bl s^{-1} during recovery exhibited complete metabolic recovery within 2 hours, whereas fish recovering in still water required more than 6 hours. Low velocity

locomotion also accelerated metabolic recovery in Atlantic salmon (*Salmo salar*) subjected to crowding stress (Veiseth et al., 2006) and coho salmon captured during commercial troll fishing (Farrell et al., 2001*b*) and gillnetting (Farrell et al., 2001*a*). However, the expedition of recovery is not universally observed (Meyer and Cook, 1996; Powell and Nowak, 2003; Suski et al., 2007; Kieffer et al., 2011), possibly as a result of variation in the species-specific response to exercise, the degree of exhaustion experienced, swimming velocity and duration of recovery, and environmental factors such as temperature (Kieffer et al., 2011). The mechanism by which low intensity exercise may aid metabolic recovery is not known, however, Milligan et al. (2000) highlight the absence of a "typical" post-exercise elevation in plasma cortisol. Cortisol is implicated in the inhibition of post-exercise glycogen restitution; suppression of cortisol release by administration of dexamethasone or metyrapone accelerates glycogen replenishment within the WM of exhausted rainbow trout, such that recovery is complete within 2 to 4 hours, compared with 8 hours in untreated fish (Pagnotta et al., 1994). Milligan et al. (2000) suggest that as free-swimming fish have lower plasma cortisol levels than those held in still water (Boesgaard et al. 1993), the elevation of cortisol following exercise is not a response to the exercise itself, but to post-exercise inactivity. During volitional swimming studies, exhausted juvenile rainbow trout demonstrated a marked preference for holding station in a current (1 bl s^{-1}), rather than seek shelter in still water; further, the preference was evident during only the first 2 hours of recovery (Lee-Jenkins et al., 2007). Brook trout also displayed a tendency to inhabit flowing (0.2-1.0 bl s^{-1}) rather than still water during recovery from exhaustive exercise (Kieffer et al., 2011). Accelerated recovery may also be encouraged through increased lactate clearance; blood flow through both the RM and WM increases during aerobic exercise (Randall and Daxboeck, 1982), presumably increasing the washout of lactic acid from the WM for subsequent use as an oxidative substrate at sites such as the red myotomal, cardiac and ventilatory muscles, where metabolic demand increases as a requirement of supporting locomotor activity (Milligan and Girard, 1993; Milligan et al., 2000). However, lactate oxidation during aerobic "cool-down" exercise has been shown to impede glycogen restitution in human exercise studies (Choi et al., 1994), presumably due to the loss of carbohydrate reserves. Similarly, in trout, the effects of the loss of WM carbohydrate to other tissues have been demonstrated by hepatic ligation; post-exercise glycogen restitution is accelerated by reducing the loss of plasma lactate to hepatic gluconeogenesis (Milligan and Girard, 1993). Regardless of the mechanism involved, it is therefore possible that the early stages of a repeat U_{crit} test may facilitate metabolic

recovery. In addition, the low-velocity swimming (typically 0.4-0.5 bls^{-1}) employed during the recovery periods of many of the existing studies of repeat swimming performance may have further aided recovery.

4.4.3 Abridged U_{crit} tests and WM function

Jain et al. (1997) demonstrated that the low velocity increments of a U_{crit} test were functionally redundant, as taxing of the RM at speeds below approximately 75% U_{crit} did not contribute to the development of fatigue at U_{crit}. Thus, to further investigate the effects of exhaustive exercise on moderate to high intensity swimming capacity, whilst minimising the compromises inherent within a complete repeat U_{crit} protocol (that is, long test duration and periods of low to moderate velocity locomotion), the U_{crit} test was modified such that immediately following fatigue at $U_{crit\ 1}$, the current velocity was increased directly to 50, 60 or 75% $U_{crit\ 1}$. Recovery ratios of 0.72 were obtained in snapper where velocity was increased to 50 or 60% $U_{crit\ 1}$; where speed was increased to 75% $U_{crit\ 1}$, 5 of the 8 fish fatigued within minutes, while the remaining 3 recorded ratios of 0.77, 0.78 and 0.80.

The similarity of the recovery ratio across the three groups of snapper, despite the variation in test duration (32 and 22 minutes on average for 50 and 60% groups respectively, while 75% ranged from less than 1 to 19 minutes) suggests some fundamental limitation to swimming performance imposed by swimming at a velocity approximately 72% of the maximum critical velocity; this limitation to performance is almost certainly the recruitment of the bulk of the WM mass. If the swimming velocities attained during the repeat swimming test are considered relative to U_{crit} determined during the initial swimming challenge, the speed at which WM is recruited can be estimated. For example, for individual fish, the penultimate swimming velocity during the repeat test – which by definition is sustainable, at least in terms of prolonged swimming – was 67 $\pm$ 15% of the critical swimming velocity determined during $U_{crit\ 1}$. The velocity at which the fish fatigued, indicative of reliance on, and failure of, WM function was 84 $\pm$ 17% U_{crit}. Hence, it appears that immediately following an exhaustive swimming event, juvenile snapper are capable of sustained swimming at speeds up to approximately 67% $U_{crit\ 1}$; at a threshold speed between 67 and 84% U_{crit}, WM recruitment becomes limiting to critical swimming performance. Although no data detailing WM recruitment in snapper exists for comparison, this conclusion is consistent

with the recruitment patterns seen in salmonids, where significant WM recruitment is estimated to occur at speeds approaching 60% (Lee et al., 2003*b*), 70-80% (Burgetz et al., 1998) and 80% U_{crit} (Jones, 1982). Patterns of WM recruitment are, however, species-specific, with some species showing low levels of WM activity at sustainable swimming speeds, and others showing no WM activity until immediately prior to fatigue (Bone et al., 1978). The high degree of variation associated with the present estimate of recruitment may be in part attributable to the relatively large velocity increments used during the U_{crit} tests.

The impairment of WM function post-exercise persists for at least 105 minutes, as indicated by the reduced swimming performance of the 1 hour recovery group, where swimming performance failed shortly after WM recruitment associated with the transition to the highest speed increment, 45 minutes into the $U_{crit\ 2}$ test (i.e. 60 minute recovery + 45 minutes until WM recruitment). Further, the similarity in the magnitude of impairment between the 75%, 60% and 50% $U_{crit\ 1}$ groups suggests no significant recovery in WM function occurs within the first 30 minutes post-exercise (the duration of the 50% $U_{crit\ 2}$ test). The duration of impairment of WM function warrants further investigation, for example through the use of abridged U_{crit} tests (i.e. 60%) in combination with a wider range of recovery periods.

4.5 Conclusions

The critical swimming performance of juvenile snapper was reduced approximately 30% following an exhaustive event, presumably a result of impaired WM function, since fish were able to swim unimpeded during subsequent low to moderate-velocity challenges. This impairment persisted for at least 105 minutes after the onset of fatigue and termination of $U_{crit\ 1}$. Critical swimming performance was progressively restored, and snapper were capable of unimpeded performance in a second U_{crit} test, separated from the first by a period of 2 hours. The use of traditional, full-length U_{crit} tests may complicate the determination of repeat swimming performance following exhaustive exercise; the use of an abridged-type repeat U_{crit} test following a range of recovery periods may represent a more effective way in which to determine the duration of impairment of WM function, and hence the recovery of critical swimming speed.

CHAPTER 5

Metabolic recovery from exhaustive exercise in juvenile snapper

5.1 Introduction

The lateral musculature of fish is spatially divided into regions of red and white fibres, whose differential recruitment allows locomotion at different speeds. Low-intensity, sustained swimming is powered by the red muscle (RM), fuelled by the oxidation of lipids and some carbohydrate (Richards et al., 2002*b*). The supplementary recruitment of some white muscle (WM) at higher sub-maximal speeds may also occur in some species (Bone et al., 1978; Johnston et al., 1977; Johnston and Moon, 1980). During high intensity, burst activity, the bulk of the white musculature is recruited, and many physiological (i.e. cardiorespiratory) and biochemical (i.e. glycolytic) systems approach their limits, culminating in fatigue (Milligan, 1996). Ultimately, exhaustive exercise is associated with significant metabolic, acid-base, ion, fluid-volume and hormonal perturbations (reviewed by Wood and Perry, 1985; Wood, 1991; Milligan, 1996). In some instances, these disturbances can be such that they result in post-exercise mortality (Wood et al., 1983; van Ginneken et al., 2008), while sublethal perturbations may confer physiological or behavioural deficits, including the impairment of subsequent swimming performance or predator evasion (Farrell et al., 1998; Jain and Farrell, 2003; Ryer, 2004; Schreer et al., 2005).

5.1.1 Disturbance of metabolic and acid-base balance

With the onset of burst activity, the energy required by the rapidly contracting glycolytic muscle exceeds its rate of synthesis by oxidative reactions. The shortfall is provided initially

by the hydrolysis of PCr and ATP; following exhaustion, these reserves may be depleted by as much as 90 and 60% respectively (Milligan and Wood, 1986; Dobson and Hochachka, 1987; Schulte et al., 1992; Milligan, 1996; Richards et al., 2002*a*). The decline in muscle PCr and ATP stimulates glycogenolysis, and the net conversion of glycogen to lactate allows for continued energy production; ultimately glycogen reserves may also be depleted by up to 90%, with a concomitant accumulation of lactic acid. A marked intracellular acidosis results from the accumulation of metabolic protons (H^+_m), produced by the dissociation of hydrogen ions from both the aforementioned lactic acid and inorganic phosphate produced by the hydrolysis of ATP. A significant extracellular acidosis also occurs, and can be resolved into two components: one of respiratory origin, and one of metabolic origin. The respiratory component is due in part to increased respiration by aerobic tissues (Milligan and Wood, 1987), but results largely from an adrenaline-mediated inhibition of red blood cell HCO_3^- flux, and thus CO_2 excretion at the gills (Wood and Perry, 1985). Despite seeming maladaptive, CO_2 retention is thought to be important in the preservation of blood HCO_3^- stores for use in the regulation of the pH of vital tissues, and in stimulating hyperventilation, ensuring maximal oxygen uptake during periods of peak demand (Wood and Perry, 1985). The metabolic component results as H^+_m responsible for the acidification of the intracellular space subsequently leaks, together with lactate (although via dissociated processes), into the blood space (Wood and Perry, 1985; Wood, 1991). Metabolic acidosis may be exacerbated by proton extrusion by red blood cells (RBCs) as they attempt to maintain intracellular pH and preserve oxygen-carrying capacity (Nikinmaa and Huestis, 1984; Primmett et al., 1986; Wood, 1991).

Despite the appearance of H^+_m and lactate in the blood, the majority of both metabolite loads are retained within the muscle; however our understanding of the mechanisms of metabolite retention remains incomplete. Increased blood flow through both the red and white musculature indicates retention is not the result of perfusion limitation (Neuman et al., 1983). Rather, lactate appears to be actively retained within the muscle; the uptake of ^{14}C-labelled lactate from the blood has been demonstrated in the WM of trout (Milligan and Girard, 1993), plaice (Batty and Wardle, 1979) and winter flounder (Girard and Milligan, 1992), despite unfavourable concentration gradients. Further, administration of the anion-transport inhibitor, 4-acetoamido-4'-isothiocyanstilbene-2-2'-disulphonic acid (SITS) increases lactate efflux, presumably by inhibiting reuptake (Turner and Wood, 1983; Sharpe

and Milligan, 2003). Attempts to further characterise the transporter responsible have yielded contradictory results, but the emerging picture is that uptake is facilitated by a carrier exhibiting monocarboxylate transporter (MCT)-like properties, recovering lactate lost via passive diffusion (Wang et al., 1997; Laberee and Milligan, 1999; Sharpe and Milligan, 2003). The mechanism of H^+_m flux is less clear; efflux from the myotome is "equilibrium-limited," and is reduced on acidification of the blood (Holeton and Heisler, 1983; Turner and Wood, 1983); however, it remains unclear whether this is a passive process, or if protons are actively extruded (Wood, 1991; Wang et al., 1994*a*).

5.1.2 Recovery of metabolic status within the WM

Post-exercise, the regeneration of PCr, ATP and glycogen, the clearance of lactate and restoration of pH balance may be key to the reinstatement of burst swimming capacity (Jain et al., 1998; Lee-Jenkins et al., 2007; Kieffer et al., 2011). The key fates of lactate and the reactions involved in the resynthesis of glycogen are illustrated in Figure 5.1. There is considerable evidence that post-exercise, glycogen is resynthesised *in situ*, with lactate as the primary substrate: in addition to >80% of the lactate load being retained within the muscle (Turner et al., 1983; Milligan and Wood, 1986; Pagnotta and Milligan, 1991), *in vivo* lactate turnover rates are insufficient to account for the observed clearance of lactate from the muscle (Milligan and McDonald, 1988), which is loosely coincident with glycogen resynthesis (Milligan, 1996). In addition, ^{14}C-lactate injected into both the muscle and blood is directly incorporated into muscle glycogen (Batty and Wardle, 1979; Girard and Milligan, 1992; Milligan and Girard, 1993). Further, fish WM has a low capacity to utilise exogenous glucose (Pagnotta and Milligan, 1991), a result of low levels of expression of glucose transport proteins (Legate et al., 2001), and low hexokinase activity (Knox et al., 1980; Storey, 1991), discounting *ex situ* (i.e. hepatic) gluconeogenesis as a means of glycogen resynthesis. The specific route by which glycogenesis takes place has yet to be fully characterised; in particular, the mechanism by which phosphoenol pyruvate (PEP) is produced from lactate remains unclear (Milligan, 1996). In hepatic tissues, the reaction is catalysed by pyruvate carboxylase (PC) and PEP carboxykinase (PEPCK), however, neither enzyme is detectable in fish skeletal muscle (Cowey et al., 1977; Knox et al., 1980). The

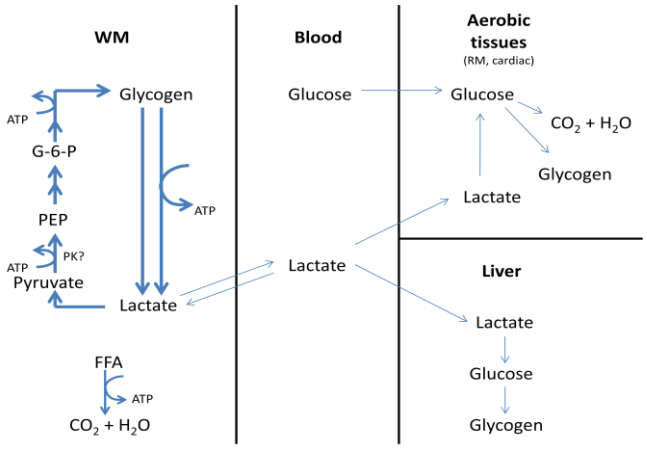


Figure 5.1 Schematic summary of lactate and glycogen dynamics during and after exhaustive exercise in fish. Retention of lactate within the white muscle (WM) is a strategy for conserving carbohydrate, by minimising potential loss to other tissues. Width of arrows indicates relative magnitude of the pathway. FFA = free fatty acids. G-6-P = glucose-6-phosphate. Modified from Milligan (1996).

reversal of pyruvate kinase (PK) has been suggested as an alternative mechanism; although *in vivo* conditions usually confer an equilibrium position favouring the production of pyruvate, Dyson et al. (1975) found that PK isolated from rabbit skeletal muscle had a reverse activity of 2% of the forward reaction rate. PK activity in fish skeletal muscle is such that a reverse reaction rate as low as 0.05% of the maximum forward reaction rate could facilitate sufficient PEP production to fuel glycogenesis (Moyes and West, 1995). The retention and *in situ* metabolism of lactate is thought to represent a carbohydrate-sparing strategy, allowing a more efficient and complete restitution of glycogen, as the release of lactate to the blood renders it vulnerable to utilisation by other tissues, and hence loss to the glycogen pool. Of the lactate released to the blood, 20-30% is subsequently oxidised by other tissues, especially the red and cardiac muscle and the liver (Milligan and Girard, 1993).

While WM is traditionally considered to have a low capacity for the storage and oxidation of lipids, there is growing evidence to suggest that the oxidation of fatty acids plays a prominent role in the provision of the ATP required to drive glycogenesis; reductions in WM free carnitine and total lipid concentrations, together with increased levels of acetyl carnitine and acetyl CoA are indicative of lipid-fuelled oxidative phosphorylation (Milligan

and Girard, 1993; Wang et al., 1994*a*; Richards et al., 2002*a*). Extracellular lactate may also represent an important glycogenic fuel (Kam and Milligan, 2006); together, this use of lipids and extracellular lactate spares intracellular lactate for use as a glycogenic substrate.

5.1.3 Disturbance of ion and fluid volume homeostasis

Changes in metabolic and acid-base status are associated with changes in ion and fluid volume homeostasis, with the disturbance being most profound at the intracellular-extracellular boundary (Wood, 1991). Increased intracellular osmolarity, as a function of elevated lactate concentration, facilitates the movement of water from the extracellular to the intracellular space, with a concomitant haemoconcentration (Turner et al., 1983; Milligan and Wood, 1986; Wood 1991). Significant movement of Na^+ and Cl^- into the tissue also occurs, concurrent with a small efflux of K^+ (Turner et al. 1983; Milligan and Wood, 1986). Whilst the location(s) of these movements is unknown, it is thought that they are associated with the regulation of acid-base balance, similar to the catecholamine-mediated regulation of the intracellular pH (pH_i) of RBCs, via Na^+/H^+ and Cl^-/HCO_3^- exchange (Nikinmaa and Huestis, 1984; Primmett et al., 1986; Wood, 1991). RBC ion flux may account for less than 10% of the net ion shifts observed post-exercise, however given the relative mass of the WM, and the degree of acid-base disturbance it experiences during exhaustive exercise, a similar mechanism need only operate to a small extent in order to account for the balance of the observed ion shift.

Ion and water flux also occurs at the gills as a consequence of changes in permeability and surface area associated with increased oxygen-diffusive capacity (Wood and Perry, 1985; Wood, 1991). In marine fish, there is a net influx of major ions and an efflux of water; the opposite is true of freshwater fish (Wood, 1991).

5.1.4 Objectives

Exhaustive exercise has the potential to cause significant metabolic, acid-base, ionic and hormonal perturbations in fish. The severity of these disturbances, and the capacity of a fish to recover from them, has significant implications for the ability of the animal to perform key

activities or behaviours following the exhaustive event, and may ultimately influence survival. Further, the magnitude of, and recovery from these perturbations may be of commercial relevance, as the pre-harvest condition of a fish is known to significantly impact product quality (Huss, 1995; see Chapter 1). Whilst changes in plasma lactate and cortisol concentrations have previously been studied in snapper (*Pagrus auratus*) in response to different capture techniques (Pankhurst and Sharples, 1992; Lowe et al., 1993), the aims of the present study were to provide a more comprehensive overview of the physiological response of snapper to exhaustive exercise, including the measurement of haematological, metabolic, acid-base and hormonal parameters. Further, the recovery profiles of any disturbances were determined over the subsequent 24 hour period.

5.2 Materials and Methods

5.2.1 Exercise and recovery protocol

Fish (75.7 $\pm$ 2.6 g) were exercised to exhaustion in an 80 l Blazka-style swimming flume, according to the U_{crit} protocol outlined in Section 2.3. Individual fish were terminally sampled immediately following exhaustion (0 h) and 0.5, 1, 2, 4, 6, 12 and 24 hours post-exercise (each n = 6) for the determination of blood and tissue biochemistry. Fish were euthanized with an overdose of MS-222, followed by spinal cord ablation (post blood sampling). Fish sampled immediately following exhaustion were placed directly into a solution of 150 mg l^{-1} MS-222 after removal from the flume; fish were otherwise placed into individual 30 l, aerated tanks for the allocated recovery period. Recovery tanks were housed within a controlled environment facility, where the air temperature was maintained at 18 ^{o}C, with a 24 hour light : 0 hour dark lighting regime. Fish were left undisturbed, and the room was only entered as required to sample fish. At the appointed time, a concentrated solution of MS-222 was added to the tank to give a final concentration of 150 mg l^{-1}; the solution was introduced into the upwelling caused by the aeration to ensure adequate mixing. Following the cessation of all movement (approximately 2 minutes), fish were prepared for sampling.

A control group of "rested" fish, not subject to any exercise protocol, were carefully netted and transferred to the recovery tanks, where they were sampled after 24 hours.

5.2.2 Blood sampling

After weighing and measuring the fish, blood was drawn by caudal vein puncture using a heparin-rinsed syringe (Terumo 1 ml syringe, ½ inch, 21 gauge needle). Subsamples of whole blood were set aside for the immediate analysis of haematocrit (Hct; 10 µl) and haemoglobin concentration ([Hb]; 5 µl). The remaining blood sample was centrifuged at 1000 *g* for 5 minutes and the plasma isolated; 95 µl was taken for the immediate analysis of plasma pH and lactate and glucose concentrations. The remaining plasma was frozen in liquid nitrogen and subsequently stored at -80 ^{o}C for the later analysis of cortisol and sodium concentrations.

All plasma samples were analysed within 16 weeks of being taken.

5.2.3 Tissue sampling

The right-hand side of the fish was gently de-scaled, and small sections of white muscle removed from two areas: the D1 block (the first uniform muscle block dorsal to the fillet centre line, slightly posterior to the pectoral fin) and the tail area rear of the anal fin, dorsal to the centre line (Fig. 5.2). Samples from the two sites were designated MD1 (muscle from D1) and MT (muscle from tail) respectively. The skin and superficial muscle layer were removed to ensure no red muscle was present in the sample. The two sites were chosen for sampling as MD1 represents the preferred site of muscle sampling by staff at Plant and Food Research, and was therefore the location of muscle samples obtained from trawl-caught fish (see Chapter 6); sampling of muscle at this site during the present study therefore allowed the direct comparison of metabolite status within the WM of trawl-caught and lab-exercised snapper. However, as the power generated by the muscle during swimming may vary along the length of the fish (Rome et al., 1993), we hypothesised that the muscle in the MT location may be more involved in powering high-intensity locomotion, and hence incur a greater metabolic disturbance.

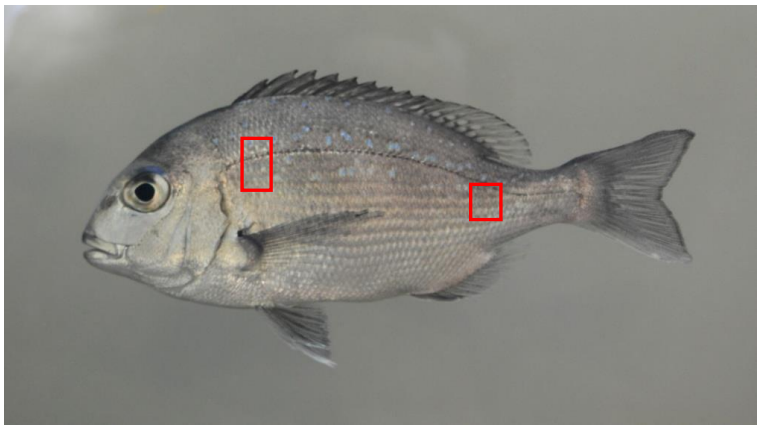


Figure 5.2. Representation of the locations of MD1 (left) and MT (right) sites from which samples of white muscle were taken for metabolite analysis. Photo courtesy of Matt Walters.

Samples of liver tissue were subsequently dissected out.

All tissue samples were promptly frozen in liquid nitrogen, and stored at -80 ^{o}C for later analysis of lactate, glucose and glycogen concentrations. Samples were analysed within 16 weeks of being taken.

5.2.4 Sample analysis

5.2.4.1 Haematocrit and haemoglobin analysis

A 10 µl microcapillary tube was filled with a sample of whole blood, sealed, and placed into a Heraeus Sepatech haemofuge, where it was spun at 9700 *g* for 5 minutes. Hct was then calculated as the length of the microcapillary tube occupied by the RBC pellet, relative to that of the plasma.

Whole blood haemoglobin levels were measured using the Drabkin's ferricyanide method. Drabkin's reagent (Sigma Aldrich, product D5941) was used to oxidise haemoglobin and its derivatives to cyanmethaemoglobin. The intensity of the colour of the resulting solution is proportional to the total haemoglobin concentration, which was determined by measuring absorbance at 540 nm and calculating concentration against a standard curve.

Mean cell haemoglobin content (MCHC) was calculated as the ratio of [Hb] to fractional Hct.

5.2.4.2 Plasma pH

Plasma pH was determined directly using an ABL-725 automated analyser (Radiometer Medical Aps; Brønsbøg, Denmark).

5.2.4.3 Plasma sodium concentration

Plasma sodium concentration was determined by adding 2 µl plasma to 1.4 ml deionised water. Samples were analysed using a Sherwood flame photometer, and sodium concentration calculated using a standard curve.

5.2.4.4 Plasma cortisol concentration

Plasma cortisol concentration was determined using a competitive enzyme-linked immunoabsorbent assay (ELISA) similar to that described by Lewis et al. (1992), with modifications described by Iremonger (2008). Briefly, cortisol-thyoglobulin conjugate was immobilised to a microtitre plate (Falcon 3912 Microtest II, Becton Dickinson, Oxnard, CA, U.S.A.). Following a series of washes, the plasma samples and a series of standards (in cortisol-free snapper plasma, see below) were added to individual wells. Antibody solution, containing cortisol mouse monoclonal (A2) antibodies (Steraloids Inc.; Wilton, N.H., U.S.A.) was added for a 60 minute incubation period, during which the free (sample-derived) and immobilised cortisol competed for antibody binding sites. The plates were subsequently washed, and sheep anti-mouse immunoglobulin, conjugated to horseradish peroxidise (HRP), was added. Following a further incubation and wash series, a substrate solution, containing tetramethylbenzidine (TMB) and hydrogen peroxide was added; peroxidase and H_2O_2 together oxidise TMB (Martin et al., 1984). The reaction was terminated by the addition of 0.9M HCl to each of the wells, which reacted with the remaining TMB to produce a yellow colour. The absorbance was then read at 450 nm using a BMG Fluostar plate reader

(Offenberg, Germany), and plasma cortisol concentration subsequently calculated against a standard curve. The higher the plasma cortisol concentration, the less A2 antibody bound to the immobilised cortisol; subsequently, less HRP-conjugated immunoglobulin remained bound to oxidise TMB, resulting in a strongly coloured solution.

Differences in the cortisol-binding properties of fish plasma relative to human plasma have previously been identified (Caldwell et al., 1991; Iremonger, 2008); hence, during the present study, the ELISA was standardised to snapper plasma through the use of dexamethasone suppression. An analogue of cortisol, dexamethasone inhibits the hypothalamus-pituitary-interrenal (HPI) axis by preferentially binding glucocorticoid receptors in the pituitary, inhibiting the release of ACTH and hence of cortisol. Four fish (approximately 400 g) were set aside in a separate tank within the flow-through aquarium system, and allowed 4 days to settle prior to dexamethasone suppression; water flow was subsequently turned off to contain the system, with existing aeration in place. Dexamethasone was dissolved in 10 ml ethanol and added to the tank to give a final exposure concentration of 10 µg l^{-1}. After 24 hours, fish were anaesthetised with AQUI-S 10 at a dose of 150 ppm for 15 minutes, and blood drawn by caudal vein puncture using a heparin-rinsed syringe. Both cortisol-free plasma and experimental samples were placed on ice and transported to Canterbury Health Laboratories for analysis within 24 hours.

5.2.4.5 Lactic acid assay

Plasma samples were assayed for lactate without any further treatment. For the analysis of muscle and liver samples, approximately 200 mg of tissue was weighed out, and chilled 6% perchloric acid (PCA) added at a ratio of 1 part tissue to four parts acid. Muscle samples were homogenised on ice using an Ultra-Turrax T-25 (IKA; Selangor, Malaysia) on medium speed, for three bursts of 20 seconds; liver samples were homogenised on ice for a single burst of 20 seconds. The homogenate was centrifuged at 6700 *g* for 5 minutes. The supernatant was extracted and neutralised to approximately pH 7 using 3M $KHCO_3$, then centrifuged at 6700 *g* for 2 minutes to remove any salt precipitate. A subsample of the supernatant was then withdrawn for lactate analysis.

Lactate concentration within both the plasma and muscle extract was determined using an ABL-725 automated analyser. A subset of samples was cross-checked using an LDH-based enzymatic assay (K-LATE 07/11, Megazyme International, Ireland).

5.2.4.6 Glucose and glycogen assays

Plasma samples were assayed for glucose without further treatment using an ABL-725 analyser. Glucose and glycogen concentrations within the muscle and liver samples were determined using a modification of the method established by Keppler and Decker (1974). Approximately 200 mg of muscle or liver tissue was weighed out, and chilled 6% PCA added at a ratio of 1 part tissue to 4 parts acid. Muscle samples were homogenised on ice using an Ultra-Turrax T-25 on medium speed, for three bursts of 20 seconds; liver samples were homogenised on ice for a single burst of 20 seconds. The homogenate was neutralised to pH 5-6 with 3M $KHCO_3$, and centrifuged at 6700 *g* for 5 minutes. A 200 µl subsample of supernatant was extracted and added to 200 µl acetate buffer (pH 4.8) for the determination of background glucose. A second 200 µl subsample of supernatant was extracted and added to 200 µl acetate buffer containing 2 mg ml^{-1} amyloglucosidase, facilitating the hydrolysis of glycogen to glucose. Samples were incubated at 40 ^{o}C for 12-16 hours. Glucose concentration was similarly determined for both sets of sub-samples using an ABL-725 analyser. Glycogen concentration (as glucose equivalents) was determined by subtracting background glucose from the total glucose concentration in the amyloglucosidase-incubated samples.

Muscle glycogen levels were found to be lower than anticipated, and below the detection level of the ABL-725 analyser. Muscle samples were subsequently reanalysed using a modification of the methods of Seifter et al. (1950), Carroll et al. (1956) and Minhorst and Liebzeit (2003). As most of the samples were consumed during the initial analysis, and preliminary work for this assay confirmed that only low levels of glycogen were present in the samples, the remaining MT and MD1 samples for individual fish were combined to provide enough tissue for analysis. Approximately 250 mg of tissue was digested in 500 µl 30% KOH at 80 ^{o}C for 20 minutes. After cooling, glycogen was precipitated by the addition of 625 µl 95% ethanol, and heated for a further 5 minutes. The sample was centrifuged at

6700 *g* for 5 minutes and the supernatant removed, before the remaining pellet was resuspended in 500 µl distilled water, and again precipitated with 625 µl ethanol. After centrifugation and removal of the supernatant, the pellet was resuspended in 1.0 ml distilled water, and centrifuged. A 200 µl subsample of the supernatant was then incubated with 1.0 ml anthrone reagent (0.05% anthrone, 1% thiourea, 72% by volume H_2SO_4) at 80 ^oC for 15 minutes. Glycogen concentration was determined by measurement of the absorbance of the resulting solution at 620 nm and calculated against a standard curve; under heated, acidic conditions, glycogen is hydrolysed to glucose, which in turn forms a furfural derivative (5-hydroxymethyl furfural) that produces a blue-green complex on reaction with anthrone (Brooks et al., 1986).

5.2.5 Statistical analysis

Statistical differences in biochemical characteristics were assessed by the pair-wise comparison of post-exercise values against those of rested, control fish, using two-tailed Student's t-tests, unpaired design (Pagnotta and Milligan, 1991; Scarabello et al., 1992). This approach was taken in preference to ANOVA with subsequent post-hoc analysis, in order to avoid the significant increase in Type II error associated with correction for multiple comparisons (Perneger, 1998). Metabolite concentration in MT and MD1 samples were compared at equivalent time points using Student's t-test of paired design. In the instance that one or more of the samples for comparison failed the Kolmogorov-Smirnov test for normality, the Mann-Whitney comparison was used in place of Student's t-test. Welch's correction was applied to t-tests where data sets were found to have unequal variance (as determined using the F-test). Significance in all cases was set at $p \leq 0.05$.

5.3 Results

The mean critical swimming speed obtained by fish during the present study was 4.97 ± 0.07 bl s^{-1} (0.75 ± 0.02 m s^{-1}), consistent with that predicted by the length – U_{crit} regression calculated in Chapter 3.

5.3.1 Haematology and plasma metabolites

Exhaustive exercise caused a significant change in Hct, increasing from 26.1 ± 2.3% in rested fish, to peak levels of 38.0 ± 1.6% immediately following exhaustion ($p = 0.0016$; Fig. 5.3a), remaining elevated during the following four hours before returning to resting levels. Similarly, a transient increase in [Hb] was evident, with a 36% increase in concentration at 0.5 h compared with rested fish ($p = 0.0132$; Fig. 5.3b). [Hb] was rapidly restored, and by 1 hour post-exercise, was not different to resting levels. It is of note that although [Hb] returned to near rested values sooner than did Hct, a degree of coupling was evident between the two, the two trends mirroring each other. No significant changes in MCHC were observed in the 12 hours following exercise, although a 16% reduction in MCHC was observed at 24 hours ($p = 0.0477$; Fig. 5.3c).

Plasma pH was significantly lower at exhaustion compared with rested fish (7.14 ± 0.04 and 7.32 ± 0.02 respectively; $p = 0.004$), returning to rested levels at 6 hours (Fig. 5.4a). Plasma lactate was elevated immediately after exercise, and continued to increase during the first hour post-exercise, to a peak concentration of 15.3 ± 2.1 mmol l^{-1} - a 32-fold increase on rested levels (Fig. 5.4b). Thereafter, lactate exhibited a sharp decrease, returning to near-resting levels at 6 hours post-exercise. Plasma glucose also increased significantly following exercise (Fig. 5.4c); although only a modest increase was observed immediately following exhaustion, glucose concentration ultimately increased 2.4-fold, peaking at 14.2 ± 1.5 mmol l^{-1} at 1-hour post-exercise ($p = 0.0008$). Elevated plasma glucose persisted for much longer than did elevated lactate, returning to resting levels at 12 hours.

Plasma sodium concentration exhibited considerable interindividual variation across all sampling times (Fig. 5.5). No significant changes in sodium concentration were observed following exhaustive exercise.

5.3.2 Plasma cortisol

Immediately following exhaustion, plasma cortisol concentration was not different to that of rested fish; however within 30 minutes, cortisol had increased 6-fold, from 29.6 ± 11.3 ng ml^{-1} to 206.7 ± 55.9 ng ml^{-1} ($p = 0.0467$; Fig. 5.6). Cortisol concentration was especially variable in the 2 hours following exhaustion, and although the data suggest a peak concentration some 9 times that of resting levels, a significant difference was not detected at this (1 hour; $p = 0.0946$) or subsequent time points.

5.3.3 Muscle metabolites

There was no significant difference between muscle lactate concentrations at the MT and MD1 sites in rested fish ($p = 0.1707$); however, the two sites did show a difference in terms of the magnitude of lactate disturbance post-exercise (Fig. 5.7a). In MT tissue, there was a slight but non-significant increase in lactate over the first half-hour post-exercise, with a tendency to decline thereafter; lactate was significantly reduced at 12 hours. In MD1 tissue, lactate concentration showed a similar trend, although the magnitude of changes appeared less than for MT tissue, and changes were not deemed significant at any time-point. MT tissue also had a tendency to higher lactate concentrations than MD tissue, with significant differences between the two sites at 0, 0.5 and 2 hours post-exercise.

Similarly, there was no difference in glucose concentration in MT and MD1 tissues obtained from rested fish ($p = 0.3204$), yet the two sites exhibited different responses to exercise (Fig. 5.7b); again the disturbance seemed to be greater at the MT site. MT glucose progressively increased during the first 6 hours of recovery, to a peak concentration of 2.27 ± 0.38 µmol g^{-1}, before returning to resting levels by 12 hours. MD1 glucose peaked at 1.27 ± 0.25 µmol g^{-1} at 2 hours post-exercise, after which time concentrations were not different to those of rested fish.

Immediately following exhaustion, muscle glycogen was reduced 78% ($p = 0.0110$) compared with resting levels (1.48 ± 0.45 µmol g^{-1}). There was no indication of glycogen resynthesis within the 24 hours post-exercise (Fig. 5.7c).

5.3.4 Liver metabolites

Liver lactate significantly increased following exercise, peaking immediately after exhaustion at 2.30 ± 0.53 μmol.g^{-1} and remaining elevated until 2 hours into recovery (Fig. 5.8a). Both liver glucose and glycogen showed considerable variation between individuals, and no significant changes resulting from exercise were detected (Fig. 5.8b, c).

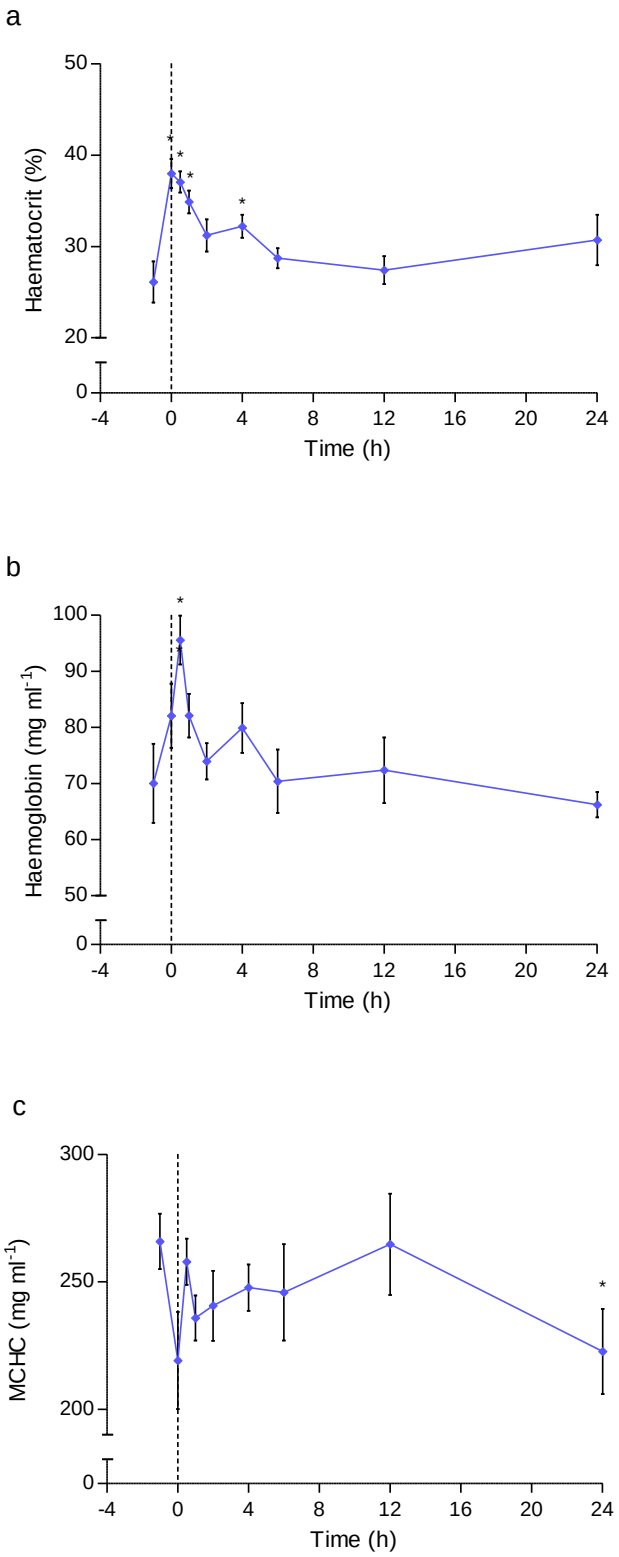


Figure 5.3. Changes in a) haematocrit, b) haemoglobin concentration and c) MCHC prior to, and during recovery from exhaustive exercise. Dashed vertical line indicates the exhaustive event; time <0 indicates values for rested fish. n = 6 at each time point. * indicates a significant difference from the resting value.

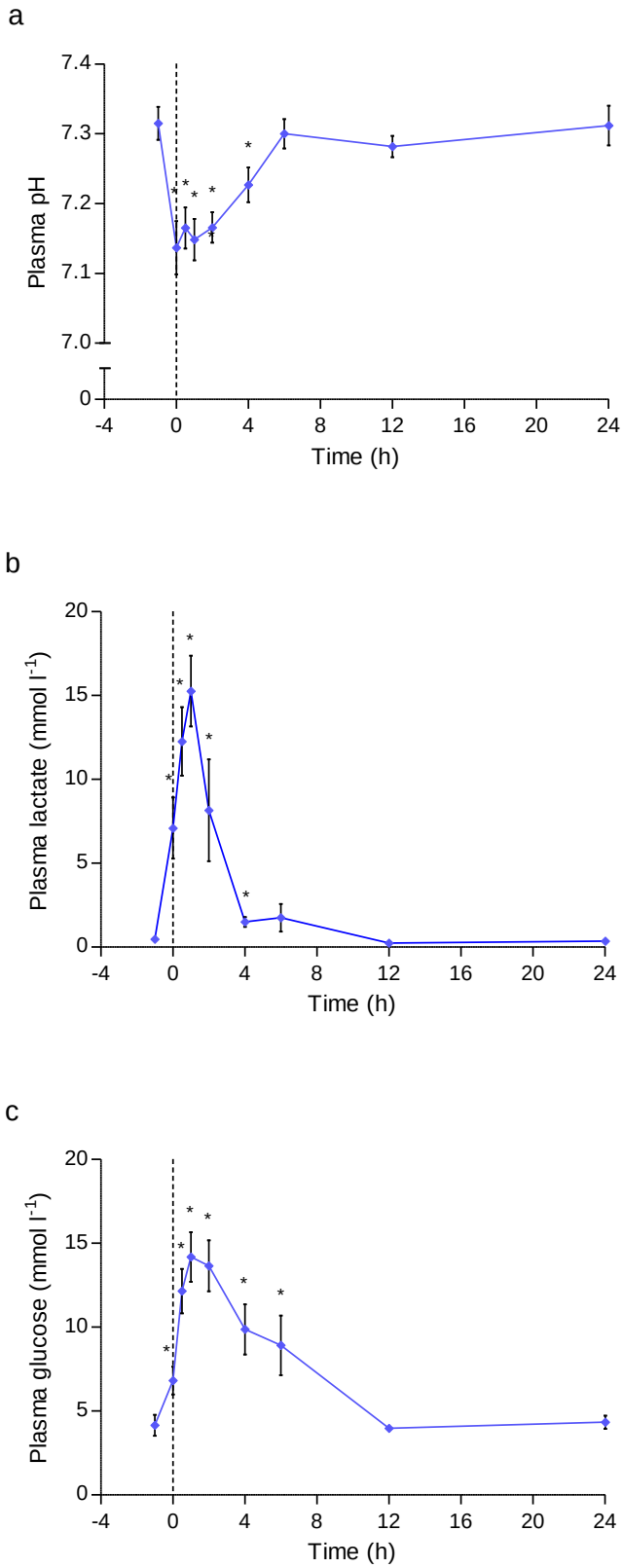


Figure 5.4. Changes in plasma a) pH, b) lactate and c) glucose concentrations prior to, and during recovery from exhaustive exercise. Other details as per Fig. 5.3.

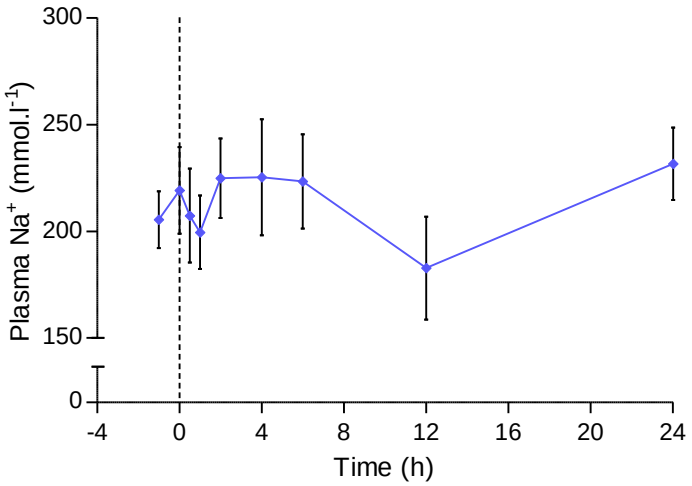


Figure 5.5. Changes in plasma sodium concentration during recovery from exhaustive exercise. Other details as per Fig. 5.3.

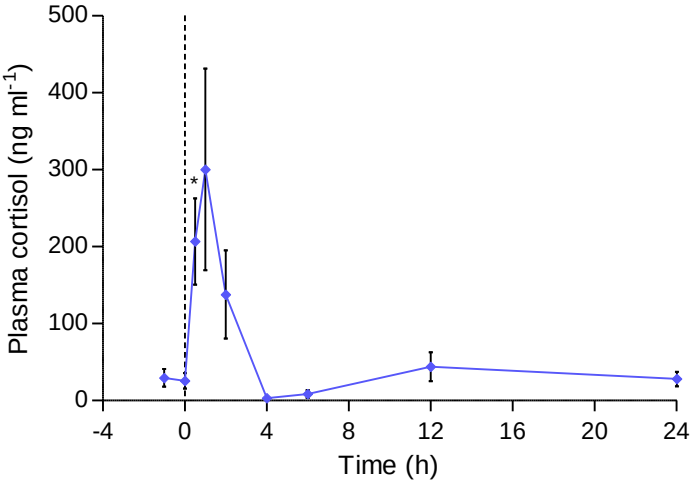


Figure 5.6. Changes in plasma cortisol concentration prior to, and during recovery from exhaustive exercise. Other details as per Fig. 5.3.

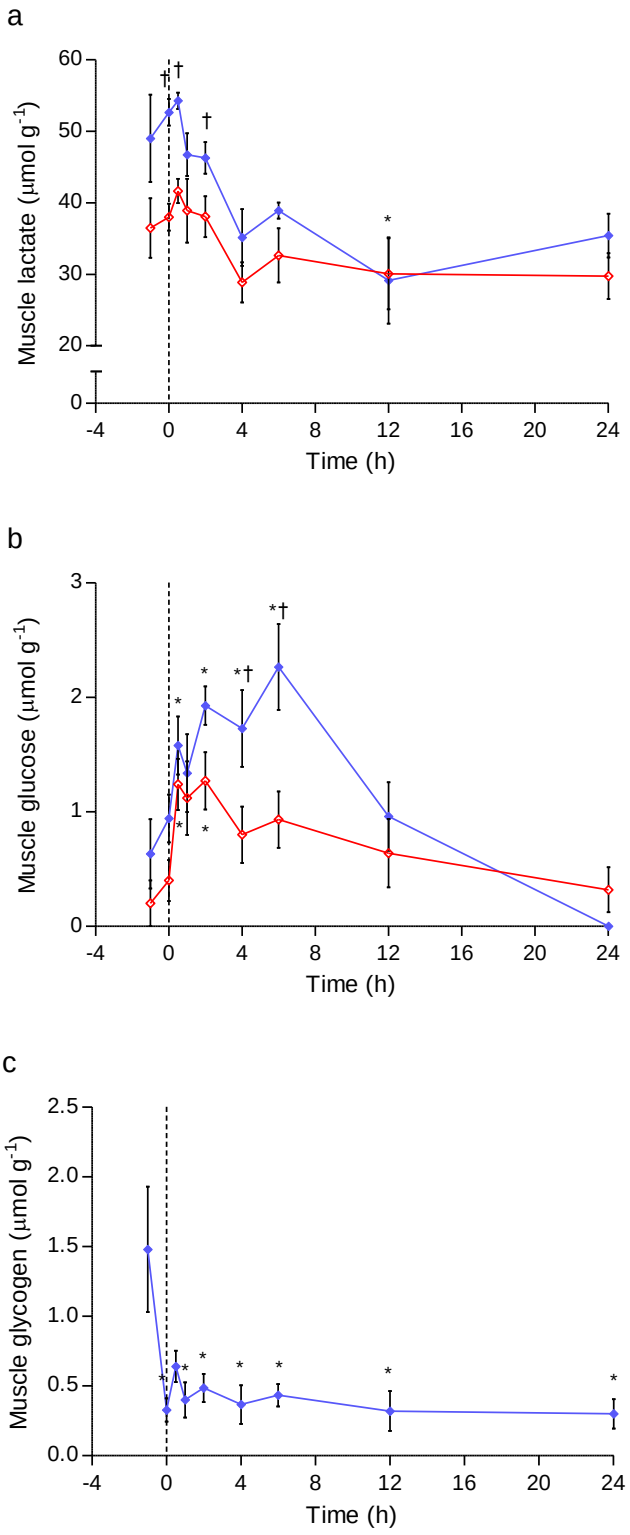


Figure 5.7. Changes in a) lactate, b) glucose and c) glycogen concentrations in the white muscle, prior to and during recovery from exhaustive exercise. * indicates a significant difference from the resting value. † indicates a significant difference between MT (◆) and MD1 (◇) concentrations at the same time post-exercise. Other details as per Fig. 5.3.

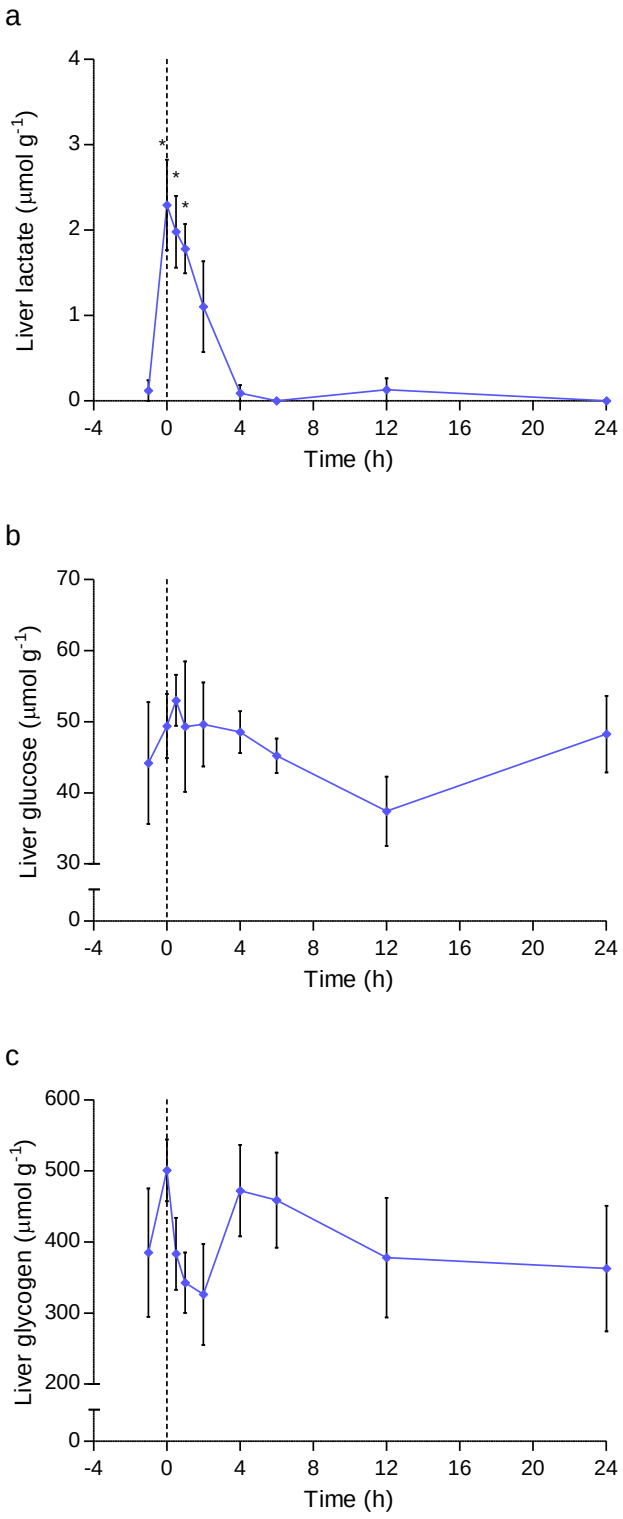


Figure 5.8. Changes in a) lactate, b) glucose and c) glycogen concentrations in the liver, prior to and during recovery from exhaustive exercise. * indicates a significant difference from the resting value. Other details as per Fig. 5.3.

5.4 Discussion

The physiological response of fishes to exhaustive exercise has been well studied, and the general response is now well-defined (Wood and Perry, 1985; Wood, 1991; Milligan, 1996; Kieffer, 2000). Within this wealth of literature, resting metabolite concentrations, the magnitude of exercise-induced perturbations, and the period of time required for their correction can vary significantly between studies, even where the same species is involved. This variation likely results from differences in exercise and sampling methodologies, recovery conditions, fish species and size, nutritional status and/or temperature (Pagnotta and Milligan, 1991; Milligan, 1996; Kieffer, 2000). The present study is the first to simultaneously characterise haematological, metabolic and hormonal responses of snapper to exhaustive exercise, and to monitor the timeframe required for their recovery. The profile of physiological perturbations observed is largely typical of that reported for other strong-swimming species (especially salmonids; Wood et al., 1983; Milligan and Wood, 1986; Pagnotta and Milligan, 1991; Schulte et al., 1993; Milligan and Girard, 1993; Pagnotta et al., 1994; Wang et al., 1994*a*; Knudsen and Jensen, 1998; Richards et al., 2002*a*), the notable exception being the lactate and glycogen profiles within the white muscle (WM); possible reasons for this discrepancy are explored in Section 5.4.3, and supplemented in Appendix 3.

5.4.1 Haematology and acid-base status

An abrupt plasma acidosis was observed following exhaustive exercise, consistent with that typically observed in fish following an exhaustive swimming event – the nadir in pH occurring immediately post-exhaustion and returning to rested levels after 6 hours. In the present case, the acidosis was comparatively modest – an average of 0.16 pH units, where changes of 0.3 to 0.7 units are typically reported (Milligan and Wood, 1986, 1987; Schulte et al., 1992; Pagnotta et al., 1994; Wang et al., 1994*a*; Knudsen and Jensen, 1998). The small magnitude of this change may be attributed to the low resting pH (i.e. compared with Wells and Dunphy, 2009; Cook and Herbert, 2012), although it is of note that plasma pH recovered to a plateau equal to resting levels post-exercise. The timeframe required for the correction of pH implies that despite its magnitude, the acidosis was physiologically significant, with the above-mentioned studies demonstrating resolution of the acidosis over a similar period of

time (Milligan, 1996). No attempt was made to resolve the acidosis into its metabolic and respiratory components.

Exhaustive exercise also resulted in the release of a significant lactate load to the blood, similar in magnitude and duration to that observed in rainbow trout (*Oncorhynchus mykiss*), which typically peaks 1-2 hours post exercise at levels of 15-20 mmol l^{-1} (Wood et al., 1983; Milligan and Wood, 1986; Pagnotta and Milligan, 1991; Milligan and Girard, 1993; Pagnotta et al., 1994; Wang et al., 1994; Milligan et al., 2000). That the peak lactate load developed over a different timeframe to that of the acidosis partly reflects their different metabolic and respiratory origins; the respiratory component of the acidosis is typically considered to account for a significant portion of the acute depression of blood pH post-exercise (Wood and Perry, 1985). In addition, the movements of H^+_m and lactate into the extracellular fluid (ECF) are governed by different mechanisms of transmembrane flux. Little is known of the route(s) of H^+_m flux, and it remains unclear whether the process is passive or if H^+_m are actively extruded from the WM (Holeton and Hiesler, 1983; Turner and Wood, 1983; Wood, 1991; Wang et al., 1994*a*). In contrast, lactate is actively retained within the WM; whilst the rate at which lactate passively leaks from the WM increases post-exercise (i.e. ten-fold in coho salmon (*Oncorhynchus kisutch*); Milligan and McDonald, 1988), the development of the blood lactate load occurs slowly, as a function of the degree by which leakage exceeds reuptake. Of the lactate that is released to the blood, an estimated 30-40% is subsequently oxidised by red muscle and cardiac tissues, with the bulk of the remainder taken up by the WM for use as glycogenic substrate and/or fuel (Milligan and Girard, 1993; Kam and Milligan, 2006).

Haematocrit, haemoglobin and MCHC observed in the present study are consistent with values reported for rested snapper by Cook and Herbert (2012); (Hct 26%, [Hb] 58 g l^{-1}, MCHC 242 g l^{-1}). Following exhaustive exercise, Hct and [Hb] increased 46 and 38% respectively, and were similar to values reported by Wells and Dunphy (2009) for snapper caught by hand-lining (Hct 38%, [Hb] 104 mg ml^{-1}, MCHC 282 mg ml^{-1}). Increased Hct, and especially [Hb], are suggestive of either increased erythrocyte circulation or significant haemoconcentration (Wood and Perry, 1985); the absence of an appreciable change in plasma [Na^+] indicates against the latter. The recruitment of additional erythrocytes from the spleen has been well documented in response to exercise (Yamamoto et al., 1980; Yamamoto, 1987,

1988; Kita and Hazawa, 1989; Wells and Weber, 1990; Pearson and Stevens, 1991), with the release of up to 93% of splenic reserves increasing the oxygen carrying capacity of the blood (Yamamoto, 1988). The injection of physiologically relevant concentrations of adrenaline and noradrenaline into the splenic artery stimulated contraction in the *in situ* spleen of Atlantic cod (Nilsson and Grove, 1974), implicating the elevated levels of circulating catecholamines post-exercise in mediating erythrocyte recruitment. Direct sympathetic stimulation of contraction via the splanchnic nerve may also contribute to splenic contraction during exercise (Nilsson and Grove, 1974; Fänge and Nilsson, 1985; Pearson et al., 1992). Increased erythrocyte concentration may also serve to increase the buffering capacity of the blood (Wood and Perry, 1985).

Increased Hct may also arise through erythrocytic swelling, indicated by a reduction in MCHC. Although not measured during the present study, blood PCO_2 may increase 50-400% after exhaustive activity (Wood and Perry, 1985), resulting from increased production by aerobically respiring tissues, inhibited excretion at the gill, and titration of blood HCO_3^- by H^+_m. Ready diffusion of CO_2 into RBCs and a resultant drop in pH_i potentially compromises the blood's oxygen carrying capacity, by virtue of the Bohr and Root effects. Adrenergic stimulation of Na^+/H^+ exchange in the RBC membrane results in the net efflux of protons in an attempt to mitigate changes in internal pH and preserve oxygen-carrying capacity. Concurrent HCO_3^-/Cl^- exchange offsets the effects of proton efflux on electrochemical gradients. As a result of net Na^+ and Cl^- influx, osmotic movement of water into the cell causes it to swell, resulting in increased Hct. The absence of any significant change in MCHC during the present study may suggest that any potential impairment of oxygen carrying capacity by the modest acidosis was sufficiently countered by the increased oxygen carrying and/or buffering capacities conferred by RBC recruitment.

5.4.2 Plasma cortisol and the stress response

Plasma cortisol concentrations in unstressed, rested fish are generally less than 30-40 ng ml^{-1} (Barton and Iawama, 1991), although Pankhurst and Sharples (1992) and Lowe et al. (1993) report concentrations <10 ng ml^{-1} for wild snapper. Whilst severe and prolonged stress may result in plasma cortisol levels of several hundred ng ml^{-1} (see Barton and Iwama,

1991), most forced-exercise protocols result in levels of 100-200 ng ml^{-1}(Pagnotta et al., 1994; Milligan et al., 2000; Milligan, 2003). Peak cortisol levels observed during the present study were higher than those previously reported for snapper caught by longline (58 ng ml^{-1}) or trawl (42 ng ml^{-1}) (Pankhurst and Sharples, 1992), and in snapper exercised using the chase method over a 1 hour period (67 ng ml^{-1}) (Lowe et al., 1993); however, these studies likely underestimate peak cortisol concentrations as fish were either sampled over only the first 60 minutes post-capture – during which cortisol was increasing - or terminally following the completion of the exercise protocol.

Cortisol mediates the intermediary metabolism of fish as part of the general stress response (van den Boon et al., 1991; Mommsen et al,, 1999). Although attempts to clarify the direct metabolic effects of cortisol are often contradictory as a result of substantial variation in experimental protocols (Barton and Iwama, 1991; Mommsen et al., 1999), it is generally accepted that cortisol impacts lipid, protein and carbohydrate metabolism, with an overall hyperglycaemic effect (van den Boon et al., 1991). The mobilisation of fuel reserves as glucose provides a means to meet the increased energy demand of tissues associated with stress; the elevation of plasma glucose during the present study is consistent with the overall stress response. Given the low capacity of the WM to utilise exogenous glucose (Pagnotta and Milligan, 1991), it is likely that plasma glucose is destined for tissues such as the RM and heart (Moon, 2001). The purpose of elevated WM glucose is less clear, but may indicate that the muscle serves as a source of fuel reserves which are subsequently released to the blood. Post-exercise, elevated glucose levels persist for up to 12 hours in both the plasma and muscle; the slow clearance or "intolerance" of a glucose load is well documented in fish, and may reflect low peripheral utilisation of glucose (Legate et al., 2001; Moon, 2001), especially once the energy demands of tissues return to rested levels.

Although the regulation of glycogen metabolism within the WM is not well understood (Milligan, 2003), there is increasing evidence to suggest that the post-exercise elevation of cortisol plays a pivotal role in determining the recovery of the metabolic status of the WM. In particular, elevated cortisol appears to inhibit glycogenesis; inhibition of cortisol synthesis or release by the administration of metyrapone or dexamethasone hastens WM glycogen restitution (Pagnotta et al., 1994; Eros and Milligan, 1996; Milligan, 2003), while the administration of exogenous cortisol prevents glycogen synthesis (Milligan, 2003). Cortisol

appears to regulate the activity of glycogen phosphorylase (GPase), possibly by maintenance of the phosphorylated state initially induced by the adrenergic stimulation of cAMP cascades (Milligan, 2003; Frolow and Milligan, 2004). Since increased glycogen synthase (GSase) activity occurs in response to reduced glycogen and increased glucose-6-phosphate concentrations, the dual activation of GPase and GSase likely results in the cycling of glycogen within the muscle, inhibiting net synthesis. The stimulation of GPase by cortisol is consistent with the role of cortisol in mobilising fuel reserves, keeping the muscle "primed" for rapid energy consumption.

Milligan et al. (2000) suggest that the elevation of plasma cortisol following exhaustive activity may not be a response to exhaustive exercise itself, but to post-exercise inactivity; low to moderate velocity exercise during recovery from fatigue may reduce the rise in plasma cortisol (Milligan et al., 2000; Veiseth et al., 2006). Further, during volitional swimming studies, rainbow trout (Lee-Jenkins et al., 2007) and brook trout (Kieffer et al., 2011) exhibited a preference for swimming against a current, as opposed to seeking still water during recovery from exhaustive exercise. Similarly to the pharmacological inhibition of cortisol release (Pagnotta et al., 1994; Eros and Milligan, 1996; Milligan, 2003), the reduction of cortisol in actively swimming fish is implicated in the expedition of metabolic recovery (Milligan et al., 2000; Farrell et al., 2001*a*, 2001*b*; Veiseth et al., 2006). If the rise in cortisol is in fact an artefact of post-exercise confinement, and is therefore at odds with the behavioural response to exhaustion displayed by wild fish, it follows that the current literature regarding the recovery of metabolites post-exercise may overestimate the length of time required for their correction, and possibly the correction of other (i.e. ionic) perturbations. Future work regarding the metabolic recovery of fish post-exercise would benefit from additional work seeking to clarify the importance of post-exercise holding conditions in determining recovery timeframes.

5.4.3 Lactate and glycogen dynamics

Lactate concentrations within the WM of control fish were 8-10 fold higher than those traditionally reported for rested fish (<5 μmol g^{-1}) (Pagnotta et al, 1994; Milligan et al, 2000; Richards et al., 2002*a*; Milligan, 2003; Kam and Milligan, 2006; van Ginneken et al., 2008),

including snapper (Cook and Herbert, 2012). Conversely, glycogen levels were significantly lower than expected; concentrations of ~20 µmol g^{-1} have been previously reported in snapper (Cook and Herbert, 2012; Tuckey et al., 2012), and various studies of rainbow trout report concentrations of 6 to 34 µmol g^{-1} (Pagnotta and Milligan, 1991; Schulte et al., 1992; Pagnotta et al., 1994; Richards et al., 2002*a*; Kam and Milligan, 2006). Both lactate and glycogen concentrations were replicated using two different assay techniques (lactate: LDH-based assay and ABL-725 analyser; glycogen: amyloglucosidase- and anthrone-based assays), with standard curves confirming the functionality of the assays. Further, the relative departures of lactate and glycogen from their "expected values" appear stoichometrically linked; consumption of ~20 µmol g^{-1} glycogen could yield ~40 µmol g^{-1} lactate by glycolytic degradation. It therefore appears that the concentrations observed were true representations of metabolite status at the time of assay. Although lactate production and oxidative phosphorylation are not mutually exclusive, lactate produced under aerobic conditions is oxidised at the same rate, such that no net accumulation occurs (Wood and Perry, 1985; Wang et al., 1994*a*). In the present case, extensive departure from the expected metabolite status at rest is most likely indicative of the pervasive recruitment of glycolytic processes within the WM, requiring the near-total consumption of muscle glycogen reserves. The question that therefore follows, is what induced this metabolic depletion?

The presence of a chronic stressor within the main aquarium system is unlikely to be responsible for the observed metabolic shift, as snapper exhibited normal feeding patterns and rapid growth, the very opposite of the reduced appetite and scope for growth typically seen in chronically stressed fish (Gregory and Wood, 1999). In addition, it seems unlikely that fish would be capable of sustaining the high swimming speeds attained if the muscle was so severely glycogen depleted (Kieffer, 2000; Gingerich et al., 2010). Alternatively, it is possible that stress could result from the holding of fish within the recovery tanks, since acute stress associated with confinement has been well documented in fish (Woodward and Strange, 1991; Pankhurst and Sharples, 1992), with implications for intermediary metabolism (Mommsen et al., 1999). However, the plasma cortisol levels of rested fish are not suggestive of any such stress, and are instead consistent with those of similar studies (20-80 ng ml^{-1}; Pagnotta et al., 1994; Milligan et al., 2000; Milligan, 2003), none of which demonstrate the metabolic depletion observed in the present study. Further, snapper constrained within respirometers a fraction of the size of the recovery tanks exhibited rates of oxygen

consumption consistent with those expected on comparison with the literature (see Chapter 3). It is also unlikely that insufficient aeration and subsequent hypoxia within the recovery tanks is responsible, as the aeration rate provided was sufficient to maintain normoxic conditions during earlier respirometric studies, and the Hct and [Hb] levels observed were consistent with those documented for normoxic snapper (Cook et al., 2011; Cook and Herbert, 2012). Finally, the analysis of metabolite levels within the WM of snapper sampled directly from the main aquarium (see Appendix 3) revealed similarly high lactate and low glycogen concentrations, suggesting that the metabolic shift is not attributable to the containment of fish within the recovery tank system.

The processing of tissue samples also represents a potential site of metabolic disturbance. In particular, the efficiency of tissue extraction and the stability of metabolites are important in accurately determining metabolic status; incomplete degradation of cell membranes could compromise the release of metabolites to the medium, whilst the incomplete denaturation of enzymes and/or warming of tissue during extraction could permit continued glycolytic activity, depleting metabolic substrates (Wang et al., 1994*b*). Hence, a set of additional experiments was carried out to ascertain whether this was the case (see Appendix 3). Ultimately, all of the manipulations yielded WM lactate concentrations in excess of 25 μmol g^{-1} and glycogen concentrations less than 2 μmol g^{-1}, similar to those observed in the present case, suggesting that these metabolite concentrations are present at the time of the excision and freezing of the muscle samples.

Given the normality of the haematological and plasma lactate and cortisol data, yet the glycogen-deplete state of muscle tissue rapidly excised and frozen, three possible explanations may account for the observed data. Firstly, it may be that snapper WM contains inherently high levels of lactate and low levels of glycogen. High levels of muscle lactate have previously been reported for snapper (Lowe et al., 1993; Tuckey et al., 2012; both ~20 μmol g^{-1}), and the post-exercise recovery of muscle lactate to a plateau of approximately 30 μmol g^{-1} may indicate that rested levels in these fish were, in fact, high. However, the lactate concentration reported by Tuckey et al. (2012) was accompanied by glycogen levels of approximately 25 μmol g^{-1}, as opposed to the 1.5 μmol g^{-1} observed in the present study. In contrast, the measurements of Cook and Herbert (2012) conform to more "traditional" low lactate, high glycogen concentrations. It would seem counterintuitive that fish would exist in

such a metabolically-deplete state, which would presumably compromise the anaerobic capacity of the tissue; one might expect that this might manifest as poor burst swimming capacity, yet no impairment of swimming performance was observed.

Alternatively, the relative concentrations of lactate and glycogen may reflect the rapid mobilisation of energy reserves in response to an acute stress experienced immediately pre-mortem, but where there may have been insufficient time for changes to manifest within the blood. For example, it is possible that the use of anaesthetics during the present study induced a stress response, since the observations of Cook and Herbert (2012) of low lactate and high glycogen were made in the absence of anaesthesia. Indeed, some snapper did show an adverse response to the introduction of MS-222, swimming at moderate speeds around the perimeter of their tank. However, this behaviour was not universal, and further, it is difficult to envisage that the intensity of the observed behaviour could be responsible for a metabolic disturbance of the magnitude induced during severe and sustained exercise (i.e. during a typical 5-10 minute "chase" protocol; Pagnotta and Milligan, 1991; Schulte et al., 1992; Pagnotta et al., 1994; Milligan et al., 2000; Milligan, 20003). Further, Wang et al. (1994*b*) recommended the use of MS-222 to sedate fish prior to euthanasia and tissue sampling, although a much greater concentration of 0.5g l_{-1} was used to prevent struggling in fish. Finally, the WM of several fish sampled directly from the aquarium and without exposure to MS-222 were also found to be high in lactate and low in glycogen (as detailed in Appendix 3), suggesting that the metabolic status of the WM was not the result of anaesthesia

Finally, there remains the possibility that there is some element of the taking or processing of samples that has been overlooked, and which facilitated continued glycolytic activity. Given the care taken to excise and freeze tissue as quickly as possible, and to ensure they remained frozen during processing, together with the considerable discussion these results have generated, we consider this possibility to be remote.

In lieu of being able to isolate the source of the metabolic disturbance within the WM, it is difficult to draw firm conclusions as to the magnitude and/or duration of the metabolic perturbations within the muscle, or of the significance of the observed differences in metabolite disturbance between MD and MT sites. Despite the number of studies addressing the metabolic consequences of exhaustive exercise in fish, relatively few have measured both plasma and muscle metabolite concentrations concurrently, and further, only several of these

measured metabolites over a period sufficient to document complete metabolic recovery (Pagnotta and Milligan, 1991; Milligan and Girard, 1993; Pagnotta et al., 1994). Within these studies, however, plasma lactate, muscle lactate and muscle glycogen appear to return to pre-exercise levels at approximately similar times (at least within the resolution afforded by the range of sampling time points. Plasma lactate is commonly measured throughout the literature as a more straightforward alternative to the measurement of muscle lactate; given the low blood volume to muscle mass ratio of fish, changes in plasma lactate are presumed to mirror changes occurring in the muscle and hence be reflective of anaerobic effort. Thus, in the absence of reliable WM glycogen and lactate profiles, it may be appropriate to use plasma lactate to estimate the timeframes involved in the clearance of lactate and resynthesis of glycogen. In this instance, plasma lactate concentrations return to rested levels by 6 hours post-exercise, suggesting that the WM returns to a rested metabolic condition over a similar time-period. Interestingly, a post-exercise decay in muscle lactate concentration also appears to plateau near 6 hours post-exercise.

Somewhat surprising, given the indication of lactate clearance, is the absence of any recovery of muscle glycogen following exercise. Capture and handling stresses have been shown to depress muscle glycogen for up to 5 days post-capture (Suuronen et al., 1996*a*). Such depression is likely a response to a chronic elevation of plasma cortisol, facilitating glycogen cycling between intermediates, such that lactate is cleared yet no net glycogen synthesis is evident. It seems unlikely that such a mechanism is responsible in the present case, however, given that cortisol returns to pre-exercise levels 4 hours into recovery. Rather, it seems that glycogen may remain low as a result of the artefact responsible for the metabolic shift discussed above.

5.4.4 The role of the liver in exhaustive exercise

Despite both liver glucose and glycogen levels showing considerable interindividual variation, there was no indication of hepatic glycogenolysis during exercise. Similarly, liver glycogen is unchanged in trout following exhaustive exercise (Pagnotta and Milligan, 1991; Milligan and Girard, 1993) and further, functional hepatectomy by ligation of the hepatic portal vein does not affect burst swimming performance (Milligan and Girard, 1993). These

observations are consistent with the idea that the WM functions largely as a closed unit during exhaustive exercise, meeting the increased energy demand by consuming endogenous fuel reserves, particularly glycogen. Poor utilisation of blood-borne glucose by the WM (Pagnotta and Milligan, 1991) further suggests that exogenous glucose is of little importance in fuelling high-intensity muscular activity.

In contrast with other vertebrate groups (Gleeson, 1996), the hepatic Cori cycle reactions are of little physiological significance in fish during recovery from exhaustive exercise. The increase in liver lactate, also observed in exhausted trout (Pagnotta and Milligan, 1991; Milligan and Girard, 1993; Pagnotta et al., 1994), is derived from the plasma lactate load. However, rather than gluconeogenic recycling and return to the WM, liver lactate probably has a glycogenic fate, representing a loss of carbohydrate to the muscle. Hepatic ligation hastens the rate of WM glycogen restitution (Milligan and Girard, 1993), highlighting the importance of the *in situ* metabolism of glycogen as a strategy for preventing lactate utilisation by other tissues and preserving carbohydrate reserves for glycogen resynthesis.

5.5 Conclusions

The profile of physiological perturbations incurred by snapper following exhaustive exercise was similar to the "classic" response that has been well-defined in rainbow trout, including disruption of metabolic, acid-base and haematological balance. These changes were evident as an acidification of the blood, splenic recruitment of erythrocytes, and the development of a significant plasma lactate load, presumably the result of glycogenolysis and lactate production within the WM. A significant stress response was also invoked, elevating cortisol levels, and consequently, elevating plasma and muscle glucose concentrations. Despite the potential presence of an unidentified stressor compromising the reliability of lactate and glycogen concentrations within the WM, plasma lactate may serve as a proxy for estimating post-exercise lactate clearance and glycogen restitution. Together, the recovery profiles of measured parameters suggest that snapper recovered from the exhaustive event after a recovery period of approximately 6 hours, although hyperglycaemia persists through

to 12 hours post-exercise, likely a result of a “glucose intolerance” well documented in teleost fish.

CHAPTER 6

The physiological response of snapper to capture in commercial trawl gears

6.1 Introduction

The majority of capture methods used in the commercial harvest of wild fish result in the stress, injury and/or fatigue of the fish, with the particular types and severity of stressors to which fish are exposed dependent on the fishing method employed (Farrell et al., 2000; Black, 2002; Davis, 2002). During capture by trawl-based technologies, fish may experience stresses associated with exhaustive exercise, confinement, crowding, barotrauma, compression and crush injury, impact injury resulting from collision with fishing gears or with other fish, scale loss, skin damage, hypoxia and/or asphyxia (Davis, 2002; Ryer, 2004; Suuronen, 2005). The relative contributions of these different stressors to the overall physiological insult varies with differences in net configuration, tow speed and duration, catch size, landing and handling protocols, fish species and size, swimming capacity, innate behavioural traits and environmental conditions, including temperature and light intensity (Davis, 2002; Ryer, 2004; Suuronen, 2005; Broadhurst et al., 2006). Whilst the physiological response of fish to exhaustive exercise and stress has been well defined within the laboratory, and is characterised by disturbances in metabolic, acid-base, ionic and hormonal homeostasis (Wood and Perry, 1985; Wood, 1991; Milligan, 1996; Kieffer, 2000), the conditions experienced by fish during capture are substantially more severe than those of the laboratory; in particular, the interaction of multiple stressors often results in cumulative and exponentially negative impacts on the physiological status of fish (Barton et al., 1986; Wedermeyer et al., 1990; Barton and Iwama, 1991; Davis et al., 2001; Davis and Olla, 2001, 2002; Davis, 2002; Broadhurst et al., 2006; Frick et al., 2010; Gale et al., 2011).

The physiological condition of fish following capture in commercial fisheries has significant implications for fisheries management. In fish that are retained as harvested catch, the peri-mortem condition of the fish is associated with the condition of the tissues post-mortem, and ultimately with the quality of the final product – the fillet (Huss, 1995; Oehlenschlager and Rehbein, 2009). Harvesting practices that result in the stress or fatigue of the fish rapidly deplete endogenous white muscle (WM) energy reserves, accelerating autolytic processes and degrading product quality by impacting taste, texture, odour, appearance and shelf life (Wells, 1987; Lowe et al., 1993; Berg et al., 1997; Erikson et al., 1997; Sigholt et al. 1997; Jerrett et al., 1998; Thomas et al., 1999; Robb et al., 2000; Skjervold et al., 2001; Black et al., 2004; Roth et al., 2006; Bosworth et al., 2007; Tuckey et al., 2010). Trawl-based fisheries are generally considered to be amongst the most stressful of the commercial fishing methods, landing fish in poorer physical condition (Fraser et al., 1965; Turunen et al., 1994; Rotabakk et al., 2011) and yielding a lower quality product (Bjordal, 2002; Rotabakk et al., 2011) than methods such as longlining, seining or trapping.

Of perhaps greater concern, however, is the fate of non-target and juvenile fish that are caught and subsequently discarded as bycatch (Davis, 2002), and with which trawl fisheries are associated with disproportionately high catches (Kelleher, 2005). Fish may be discarded for a number of reasons, including regulations pertaining to minimum size limits, catch limits or local season closures intended to protect vulnerable stocks, or for economic reasons such as high-grading of the catch to retain the largest individuals and/or more desirable species (Davis, 2002; Diamond and Campbell, 2009). The physiological condition of these fish on their release may have profound effects on their fitness and survival. Mortality may occur as a direct result of physiological stress or injury sustained during capture (Wood et al., 1983; Olla et al., 1997, 1998; Stewart, 2008; van Ginneken et al., 2008; Olsen et al., 2012), or indirectly as a result of physiological or behavioural impairments, including compromised swimming ability (Schreer et al., 2005), predator detection and evasion (Olla et al., 1997; Ryer 2002; Ryer et al., 2004) and/or increased susceptibility to disease and infection (Suuronen, 2005; Davis and Ottmar, 2006). The fitness of fish that survive discard may be compromised through stress-induced changes in reproductive function or behaviour, reduced appetite and growth suppression or altered social function (Wedermeyer et al, 1990; Barton and Iwama, 1991; Barton, 1997; Meka and McCormick, 2005).

Quantifying mortality and fitness impairments in discarded fish is essential to understanding the full impacts of fishing activities on fish stocks, and hence to their effective management (Diamond and Campbell, 2009). Estimates of mortality in organisms captured by towed fishing gears vary between 0 and 100%, reflective of substantial differences in the species-specific response to capture, and in capture methodology and holding conditions (see Chopin and Arimoto, 1995; Broadhurst et al., 2006 for reviews); however, it is generally considered that the proportion of fish that die exceeds that which survives (Broadhurst et al., 2006). As discarded fish are often juveniles or young adults, high rates of discard mortality mimic recruitment failure; stock assessments that do not account for discard mortality may therefore overestimate the spawning biomass and set inappropriate catch limits, resulting in the depletion of stocks (Diamond and Campbell, 2009). The failure to adequately account for discard mortality has been cited as a contributing factor in the collapse of Atlantic cod stocks off the coast of eastern Canada in the 1990s (Myers et al., 1997).

An understanding of the factors that influence the overall stress response and hence the implications for survival and fitness is critical for the accurate prediction of mortality, and to coherent attempts at mitigating the inciting stressor(s) (Broadhurst et al., 2006). Physiological measurements such as plasma cortisol, glucose and lactate are often considered useful in determining the magnitude of stress experienced, and in evaluating recovery (Morgan and Iwama, 1997). The use of such indices in determining the physiological condition of fish post-capture may provide a means of predicting not only direct mortality, but also indirect mortality and reduced fitness, providing a more complete picture as to the overall impacts of fishing activities. The ability to utilise physiological indicators as predictors of fitness and survival depends upon establishing their relationship with mortality and/or ecologically-relevant fitness indicators, such as swimming performance or predator evasion (Barton, 1997; Davis et al., 2001). While some studies have demonstrated a clear association between physiological measures and mortality (Beamish, 1966; Olla et al., 1998; Davis and Schrek, 2005; Diamond and Campbell, 2009) or performance (Farrell et al., 1998; Jain et al., 1998; Jain and Farrell, 2003), others have not (Olla et al., 1992; Oddson et al., 1994; Davis et al., 2001). The correlation between physiological condition and mortality and/or fitness is yet to be fully understood.

6.1.1 Objectives

Snapper (*Pagrus auratus*)are an important recreational and commercial fish species; in the case of the latter, the catch is taken largely by longline and trawl (Pankhurst and Sharples, 1992; Ministry of Fisheries, 2006; Ministry for Primary Industries, 2013*a*). The physiological status of snapper caught by commercial fishing methods has received little attention. Snapper caught by longline and trawl fishing exhibit signs of stress and fatigue at landing, with elevated cortisol and plasma and muscle lactate concentrations (Pankhurst and Sharples, 1992; Lowe et al., 1993). Discard mortality rates in excess of 50% have been reported in snapper caught by trawl (Sumpton and Jackson, 2005) and traps (Stewart, 2008).

The preceding chapter explored the physiological perturbations that occur following a laboratory-based exhaustive exercise (U_{crit}) test; no mortality was observed, and snapper appeared capable of a rapid metabolic recovery. However, as laboratory-based studies are often criticised for their lack of realism (Davis, 2002), and in the absence of other capture-related stressors, the relevance of this data in describing the condition of snapper caught in a commercial setting is unknown. The aim of the present study was therefore to analyse the physiological condition of snapper caught during a series of trawl surveys that were operated in the same manner as a commercial tow, and to assess their potential for metabolic recovery, which may be critical to the survival and fitness of discarded fish. A comparison of the condition and recovery of snapper following laboratory-based exercise and trawl capture was made, to determine the suitability of U_{crit} swimming challenges as a means to model capture stress during future studies.

6.2 Materials and methods

6.2.1 Trawl surveys and collection of fish

The 19.8 m fishing trawler *FV Bacchante* was commissioned by Plant and Food Research for a series of trawl surveys in the Tarankai Bight in February 2012. For each trawl, a grey scraper net with 5” codend (160’ ground rope, 9” Hampidjan Albatross trawl, 57 m^2 surface area) was towed at a depth of 50 m, with a tow speed of approximately 3 knots

(~5.6 km h^{-1} or 1.5 m s^{-1}), for 2.25 to 2.75 hours. Water surface temperature was approximately 20 ^{o}C.

At the conclusion of the tow, the net was retrieved; fish were sorted and the catch composition recorded for a range of data, including species and mass. Following two of these trawl events, a number of live snapper (30 following trawl 1, 33 following trawl 2; 1122 $\pm$ 38 g) were selected from the trawl and immediately set aside into an on-deck holding system, comprising a 750 l bin with a continuous flow of fresh seawater (~75 l min^{-1}). Aeration was provided via a Heliea Aeo-007 diaphragm pump. Post-capture mortality was recorded, and a sub-sample of fish was retrieved and euthanized, and tissue samples collected at 0.7, 0.8, 4.7, 6.9, 10.5 and 17.0 hours after landing (each n = 8; see Table 6.1).

6.2.2 Collection of blood and tissue samples

Fish were individually retrieved from the holding tank with a dipnet, then rapidly killed using the traditional Japanese *ike jime* method. Fish did not show a negative response to the presentation of the dip net, and were able to be netted and killed without struggle. A blood sample was drawn by caudal vein puncture, and collected in a 3 ml plastic vacutainer pre-coated with K_2EDTA (BD, New Jersey, U.S.A.); a 5 µl subsample was set aside for the determination of haemoglobin, and the remainder of the sample centrifuged at 6000 rpm (Labnet Spectrafuge Mini, Labnet International, Inc., New Jersey, U.S.A.) to isolate the plasma. The cut surface pH of the white muscle was measured in the MD1 region using a flat surface, combination pH electrode (SensoreX); a sample of this tissue was subsequently dissected out, followed by the excision of liver tissue.

All samples were immediately placed on ice, and then frozen and stored at -20 ^{o}C whilst the vessel was at sea; on return to port the samples were packed in dry ice and shipped to UC, where they were subsequently stored at -80 ^{o}C until analysis.

6.2.3 Sample analysis

Samples were assayed as per the protocols outlined in Chapter 5. Haemoglobin concentration was determined using Drabkin's reagent. Plasma samples were assayed for

pH, and lactate and glucose concentrations directly using an ABL-725 analyser. Lactate concentration was determined for muscle and liver samples by homogenising tissues in 6% PCA, neutralising the homogenate to pH 7 with 3M $KHCO_3$, and analysing the content of the supernatant using an ABL-725 analyser. Glucose and glycogen content was determined for muscle and liver samples using amyloglucosidase incubation, based on the methods of Keppler and Decker (1974). Muscle glycogen content was found to be below the detection limits of the ABL-725 analyser, and samples were subsequently reanalysed using a modification of the anthrone-based assay of Seifter et al. (1950) and Carroll et al. (1956).

Plasma cortisol concentrations were determined using a modification of the competitive ELISA described by Lewis et al. (1992), as detailed in Chapter 5.

6.2.4 Statistical analysis

In the absence of data describing the condition of control or "rested" wild snapper, it was not possible to determine either the magnitude of the physiological insults incurred during capture or a complete recovery profile for these disturbances.

To allow the comparison of post-capture metabolite levels with those of snapper subject to a laboratory-based, incremental exercise test (Chapter 5), the data obtained during the present and laboratory-based studies were first graphically overlaid, to allow easy visual comparison of metabolite levels and recovery trends; standard errors associated with laboratory-derived data were omitted for clarity. To allow the statistical comparison of the differences in the recovery trends, despite differences in the post-exercise sampling times between the two studies (an unavoidable logistical constraint), the data from both trawl-caught and lab-exercised snapper were binned as detailed in Table 6.1. Metabolite concentrations at similar sampling times post-capture/post-exercise were subsequently compared using a two-tailed Student's t-test of unpaired design.

Post-capture mortality was observed during this study. To determine whether any of the physiological parameters measured differed between dead and surviving fish, and could therefore represent a potential indicator of impending mortality, measurements obtained from live and dead fish within each time bin were compared using a Student's t-test of unpaired design.

Table 6.1. Assignment of post-exercise sampling times to bins, to allow the statistical comparison of metabolite levels in commercially caught snapper with those of fish exercised to exhaustion using a laboratory-based exercise test.

	Sampling time (h)	
Bin label Time (h)	Laboratory-based U_{crit} (Chapter 5)	Trawl-caught fish (present data)
0	0	-
0.5-1.0	0.5, 1.0	0.7 ± 0.1 [2], 0.8 ± 0.2 [1]
2-5	2, 4	4.7 ± 0.1 [1]
6-8	6	6.9 ± 0.1 [2]
10-12	12	10.5 ± 0.1 [1]
16-24	24	18.0 ± 0.1 [2]

Superscripted numbers indicated whether the fish from respective sampling groups were caught during Trawl 1 or Trawl 2.

6.3 Results

6.3.1 Catch composition

Snapper was the dominant species caught during the trawls; gurnard, trevally, school shark, leatherjacket and John Dory were also significant contributions to the catch (Table 6.2). Due to time constraints, no attempt was made to measure the size of individual fish or to determine the relative proportion of juvenile to adult fish. There was no significant difference in the size of snapper held for sampling following the two trawls ($p = 0.9969$), nor were there differences in the size of fish comprising each time bin post-capture ($p = 0.0543$).

Table 6.2. Trawl methodology details and catch composition of two simulated commercial trawls in the Taranaki Bight. Where no mass is indicated for a species, the total yield for that species was less than 10 kg.

	Trawl 1	**Trawl 2**
Location	South Taranaki Bight	North Taranaki Bight
Trawl speed (knots)	3.0-3.2	2.7-3.2
Duration (hours)	2.75	2.25
Catch composition - species and total mass (kg)	Snapper (*Pagrus auratus*) 375kg Gurnard (*Chelidonicthys kumu*) 100kg Trevally (*Caranx georgianus*) 100kg Leatherjacket (*Parika scaber*) 25kg Eagle ray (*Myliobatus tenuicaudatus*) 20kg John Dory (*Zeus faber*) 20kg Lemon sole (*Pelotretis flavilatus*) 5kg Blue cod (*Parapercis colias*) 5kg Barracouta (*Thyrsites atun*) School shark (*Galeorhinus galeus*) Star gazer (*Genyagnus monopterygius*) Porcupine fish (*Allomycterus jaculiferus*) Rough skate (*Dipturus nasutus*) Rig (*Mustelus lenticulatus*)	Snapper (*Pagrus auratus*) 325kg Gurnard (*Chelidonicthys kumu*) 75kg Trevally (*Caranx georgianus*) 75kg School shark (*Galeorhinus galeus*) 40kg Leatherjacket (*Parika scaber*) 15kg Turbot (*Colistium nudipinnis*) 15kg Lemon sole (*Pelotretis flavilatus*) 10kg Porcupine fish (*Allomycterus jaculiferus*) 10kg John Dory (*Zeus faber*) Barracouta (*Thyrsites atun*) Star gazer (*Genyagnus monopterygius*) Rough skate (*Dipturus nasutus*) Rig (*Mustelus lenticulaus*) Numb ray (*Torpedo fairchildi*) Carpet shark (*Cepaloscyllium*)
Size of snapper held post-capture	1122 $\pm$ 48 g 38.0 $\pm$ 0.6 cm	1122 $\pm$ 58 g 38.0 $\pm$ 0.7 cm
Post-trawl mortality within holding tanks	9/33	0/30

6.3.2 Post-capture mortality

Immediate mortality – the number of fish that were dead at the time of landing or that died shortly thereafter – was not quantified; however, the majority of the catch was landed alive. It was noted that the fish landed following the second trawl appeared much more lively than those landed following the first, which were lethargic in comparison. Delayed mortality rates in snapper landed following Trawl 1 were higher than for Trawl 2; of 33 fish held following capture in the first trawl, 2 died within the first hour post-capture and a further 7 died over the following 4 hours (Table 6.2). There was no mortality in fish held for sampling following the second trawl.

Once fish were placed into the holding tank, they were left undisturbed (other than for sampling), so behaviours that may have indicated impending mortality and provided the opportunity to sample moribund fish (Beamish, 1966; Wood et al., 1983), were not monitored. As a result, 2 of the 8 fish sampled 0.8 hours post-capture, and 6 of 8 fish sampled 4.7 hours post-capture were dead at the time of sampling (a 7^{th} dead fish was not sampled). For all of the parameters described in the following sections, other than plasma lactate (see Section 6.3.4), no statistical differences in the measurements obtained from live and dead fish were evident; the data sets were therefore pooled in order maximise the number of observations included within the present dataset.

6.3.3 Physiological condition at landing and metabolic recovery

There were no significant differences in any of the physiological parameters measured between fish sampled at 0.7 and 0.8 hours following the respective trawls, data for these two groups were subsequently pooled for clarity, and are hereafter collectively referred to as fish sampled 0.75 hours post-capture.

6.3.3.1 Haematology and plasma metabolites

A significant post-capture plasma acidosis was evident in trawl-caught snapper, with pH increasing from 6.63 $\pm$ 0.03 at 0.75 hours post-capture to 6.89 $\pm$ 0.08 over the subsequent 4 hours, appearing to stabilise at a pH approximately 6.9 thereafter (Fig. 6.1a). The pH of

plasma obtained from trawl-caught fish was depressed at all time points compared with that of lab-exercised fish; subsequent work revealed that in blood samples taken from a rested snapper (sampled from the laboratory aquarium), the use of an EDTA-coated vacutainer resulted in a reduction of 0.3 pH units compared with the sample taken using a heparin-rinsed syringe (pH 6.9 and 7.2, respectively).

Post-capture changes in plasma lactate concentrations were similar to those observed in lab-exercised fish, with peak lactate loads developing within the first hour of recovery, at 15.3 + 1.0 and 15.3 + 2.1 mmol l^{-1} respectively (Fig. 6.1b). In contrast to the rapid clearance of lactate observed following U_{crit}, the plasma lactate concentration of trawl-caught fish remained elevated over an extended period, with concentrations of 13.6 + 0.1 mmol l^{-1} at 4.7 hours post-capture before slowly declining, such that by 10.5 hours, concentrations were not significantly different to those of laboratory-exercised fish at a similar time post-exercise. The elevated plasma lactate concentration of fish that suffered mortality is discussed in Section 6.3.4.

In contrast with the peak plasma glucose load that developed in the hour following fatigue at U_{crit}, the plasma glucose concentration of trawl-caught fish displayed a relatively stable, chronic elevation of 8 to 10 mmol l^{-1}over the first 10.5 hours post-capture, with indications of a decline thereafter (Fig. 6.1c).

Unfortunately, haematocrit levels could not be determined at sea. Further, haemoglobin levels could not be determined for trawl-caught fish, as the samples appeared to have desiccated during frozen storage, and were unable to be dissolved in Drabkin's solution for analysis.

6.3.3.2 Plasma cortisol

Similar to the plasma cortisol concentrations observed in the laboratory-based experiment, cortisol levels of trawl-caught fish exhibited considerable interindividual variation. No obvious trend in cortisol level was evident; there was no distinct peak evident in the hours post-capture, as observed in fish following U_{crit} (Fig. 6.2), rather, cortisol levels remained elevated through the 18 hours post-exercise, with concentrations at different sampling times fluctuating between 83.7 $\pm$ 26.0 and 227 $\pm$ 49.7 ng ml^{-1}.

6.3.3.3 Muscle metabolites

A significant intracellular acidosis was evident within the WM of trawl-caught snapper, with pH increasing linearly from 6.40 ± 0.02 at 0.75 hours post-capture to 7.61 ± 0.07 at 18 hours post-capture (Fig. 6.3).

Peak lactate concentrations within the WM were observed 0.75 hours post-capture, and were significantly higher than the corresponding levels within the MD1 tissue of lab-exercised fish, at 56.3 ± 1.7 and 38.0 ± 1.9 μmol g^{-1}, respectively (Fig. 6.4a; $p < 0.0001$). Lactate levels decayed thereafter, and by 6-8 hours post-capture, were not different to those of laboratory-exercised fish. The increase in WM lactate levels 18 hours post-capture is unexplained.

Changes in muscle glucose showed vastly different trends between lab-exercised and trawl-caught fish (Fig. 6.4b). While muscle glucose was elevated immediately following U_{crit}, peaking 1 hour into recovery and decaying slowly thereafter, glucose levels in trawl-caught snapper remained low following capture, at levels similar to those of rested laboratory fish. An increase in glucose concentration was evident at 6.9 hours post-capture, peaking at 10.5 hours at 1.98 ± 0.46 μmol g^{-1}.

In contrast, muscle glycogen concentrations demonstrated remarkable similarity between lab-exercised and trawl-caught snapper, with levels of ~0.6 μmol g^{-1} 1 hour post-capture, decreasing to <0.5 μmol g^{-1} by 6.9 hours post-capture, and showing no indication of recovery for the duration of the period monitored (Fig. 6.4c).

6.3.3.4 Liver metabolites

Similar to lab-exercised fish, trawl-caught snapper exhibited an increase in liver lactate content following capture (Fig. 6.5a). In the latter, the peak lactate load was more than double that seen following U_{crit}, and was observed 4.7 hours post-exercise, rather than immediately following fatigue. Lactate therefore remained elevated for an extended period relative to that of laboratory-exercised fish, with no significant difference evident between the two groups at 10-12 hours following fatigue.

In contrast, liver glucose and glycogen levels exhibited significantly different post-exercise responses to those seen in the laboratory. Whilst liver glucose levels were similar in

lab-exercised and trawl-caught fish in the hour following fatigue (Fig. 6.5b), glycogen levels were substantially lower in trawl-caught fish than laboratory fish (Fig. 6.5c). Both glucose and glycogen concentrations declined significantly over the subsequent 4 hours – glucose was reduced by approximately 57% and glycogen by 85% – with both metabolites remaining depressed for the duration of the sampling period.

6.3.4 Physiological parameters as indicators of mortality

The physiological condition of fish that experienced capture-induced mortality was compared with that of surviving fish. Of the physiological indicators measured during the present study, only plasma lactate behaved different between fish that died and those that did not (Fig. 6.6). In fish that suffered mortality, plasma lactate levels were lower in the first hour post-capture than for fish that survived; however, whilst the lactate concentration of surviving fish declined thereafter, lactate levels of non-survivors increased significantly, reaching 19.5 ± 0.6 mmol l^{-1}at 4.5 hours post-capture.

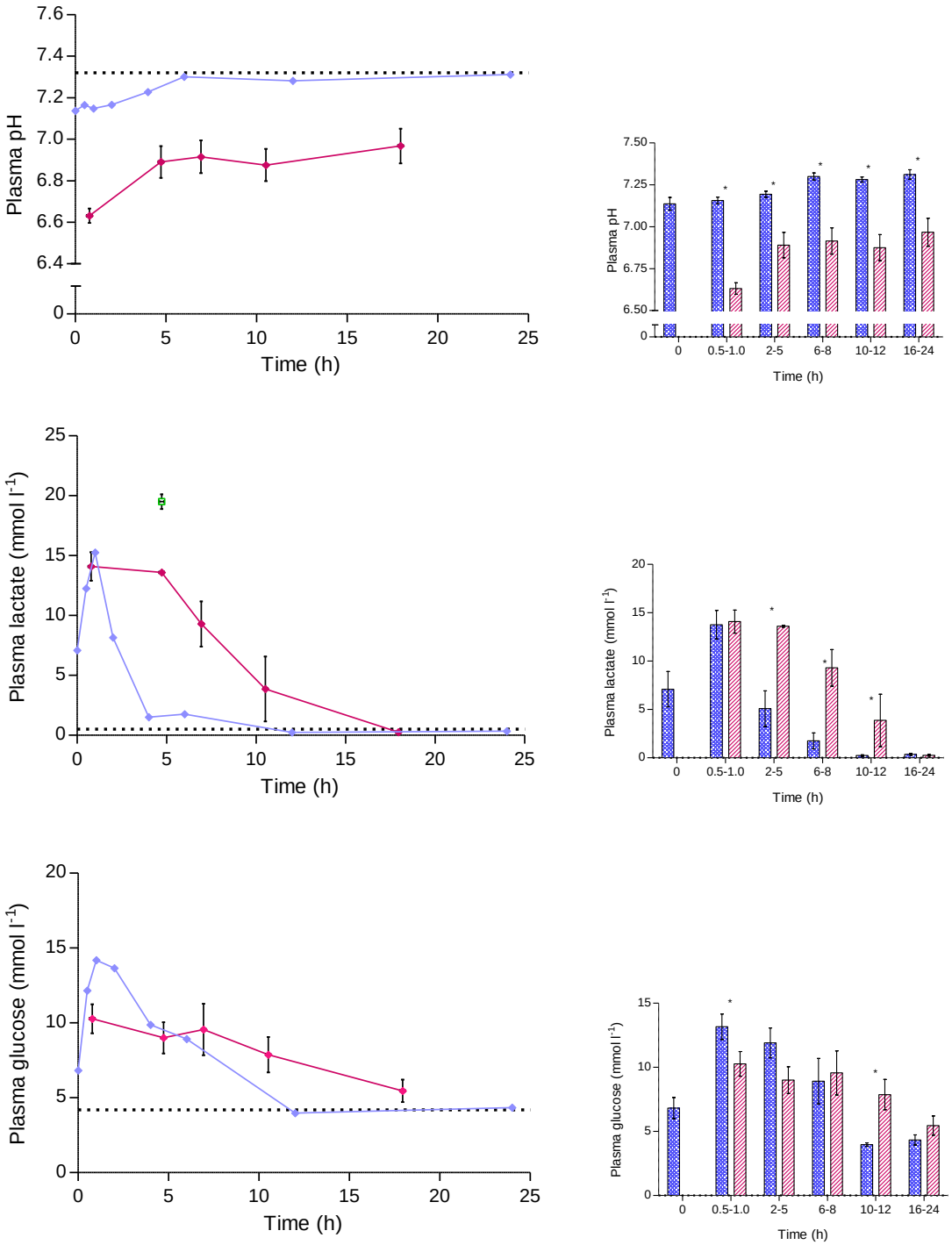


Figure 6.1. Changes in plasma a) pH, and b) lactate and c) glucose concentrations during recovery from capture in a commercial trawl net (◆); changes observed in snapper following exhaustion during a laboratory-based incremental exercise test are illustrated for comparison (◆). Note that for plasma lactate measured at 4.7 hours post-capture, n=2; the lactate concentration in snapper that suffered capture-induced mortality was found to be significantly higher of that of surviving fish, and is therefore displayed separately (□) and excluded from statistical analysis; see also Figure 6.6. Dashed horizontal lines represent the corresponding values in rested fish during the laboratory-based study. Inset: illustration of the binned recovery times that allowed statistical comparison of parameters between trawl- (▨) and laboratory -induced (▨) exhaustion. * indicates a significant difference between trawl-caught and laboratory-exercised fish within the same recovery time bin.

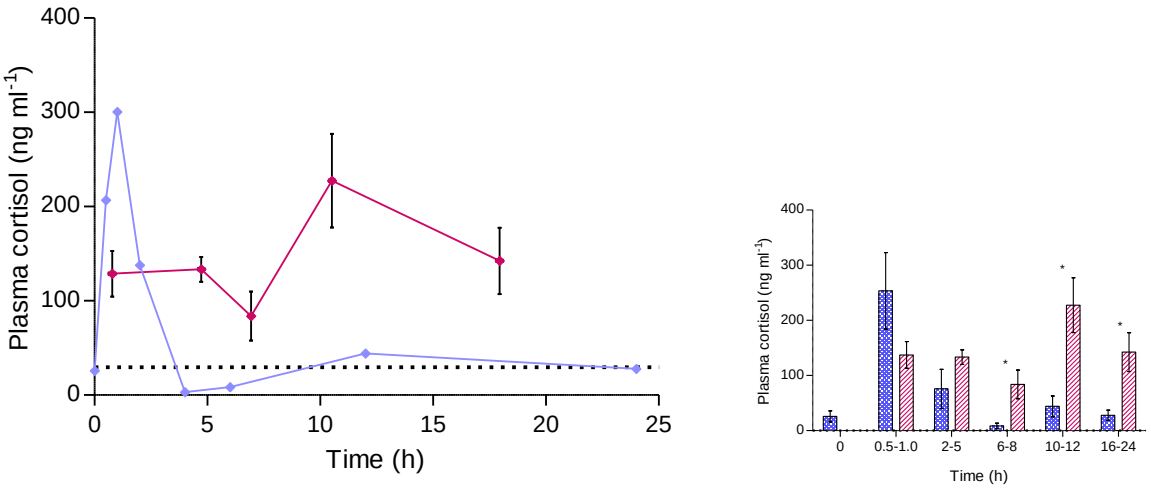


Figure 6.2. Changes in plasma cortisol concentration during recovery from capture in a commercial trawl net (♦); changes observed in snapper following exhaustion during a laboratory-based incremental exercise test are illustrated for comparison (♦). Other details as per Figure 6.1.

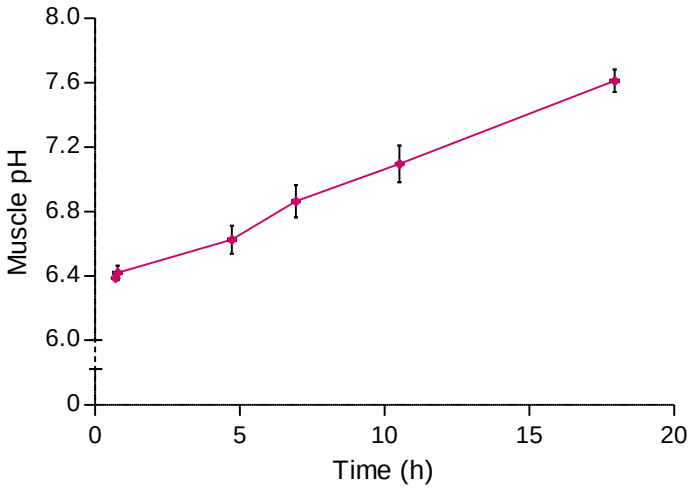


Figure 6.3. Changes in WM pH during recovery from capture in a commercial trawl.

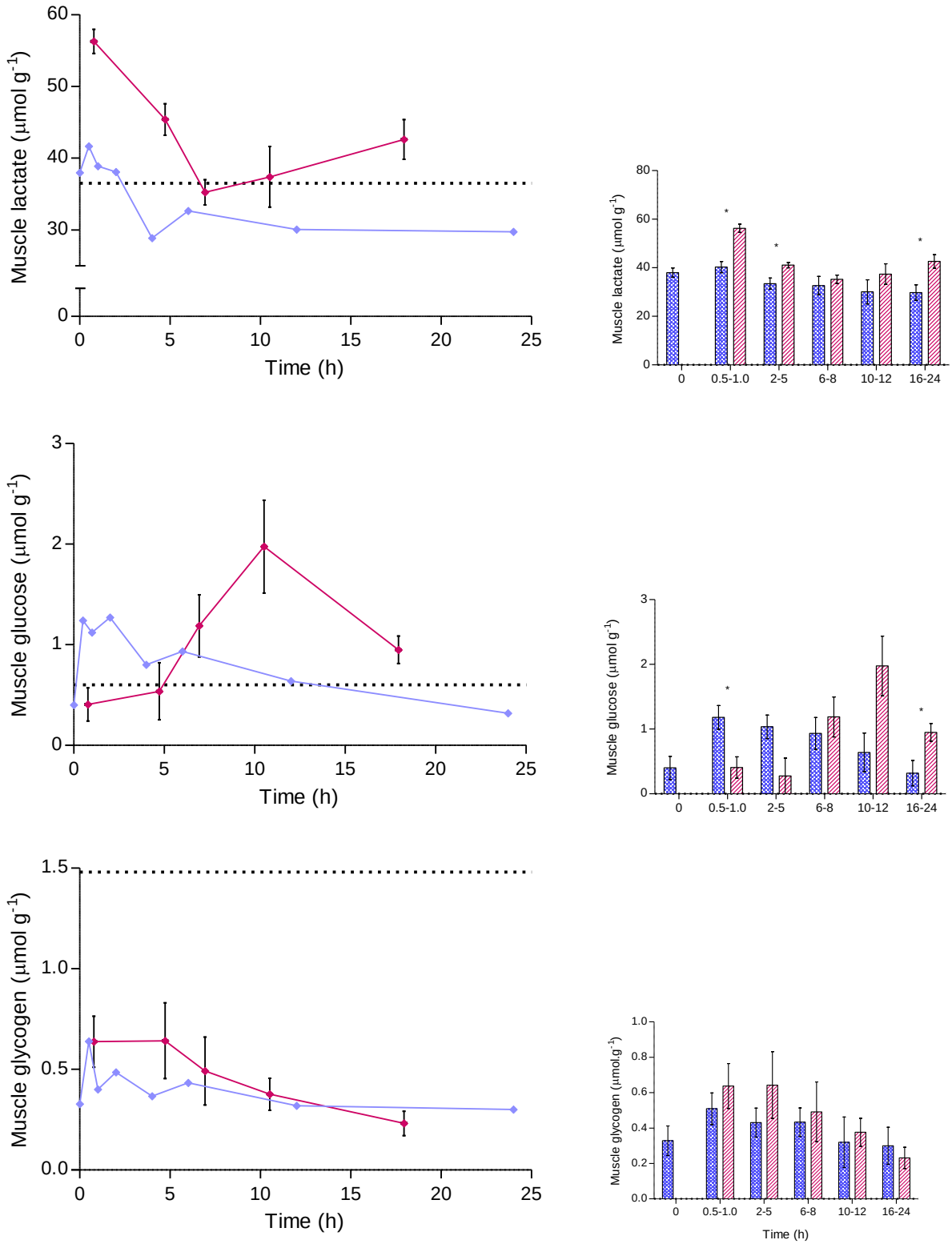


Figure 6.4. Changes in a) lactate, b) glucose and c) glycogen concentrations within the WM during recovery from capture in a commercial trawl net (♦); changes observed in snapper (WM sampled at MD1 site) following exhaustion during a laboratory-based incremental exercise test are illustrated for comparison (♦). Other details as per Figure 6.1.

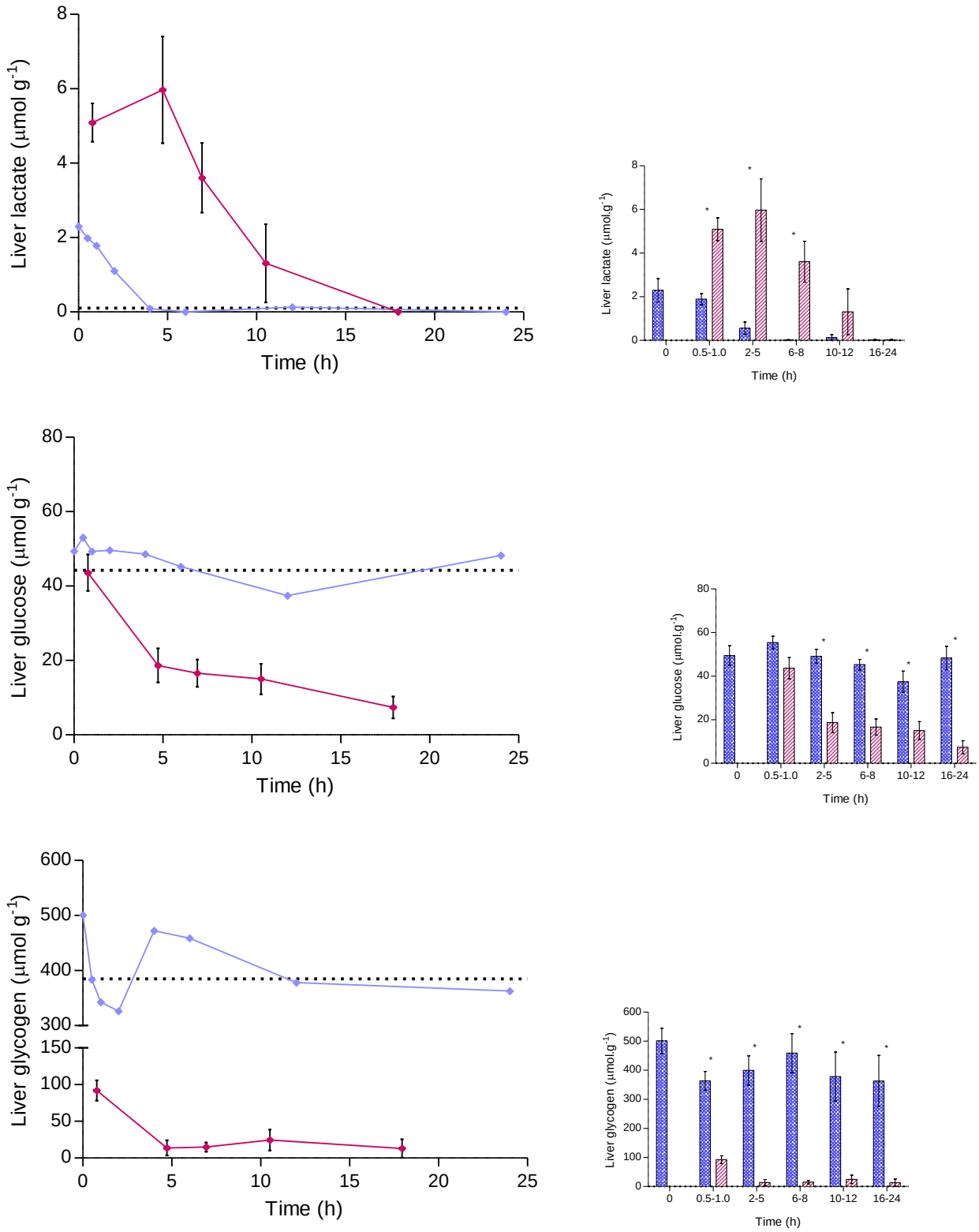


Figure 6.5. Changes in a) lactate, b) glucose and c) glycogen concentrations within the liver during recovery from capture in a commercial trawl net (♦); changes observed in snapper following exhaustion during a laboratory-based incremental exercise test are illustrated for comparison (♦). Other details as per Figure 6.1.

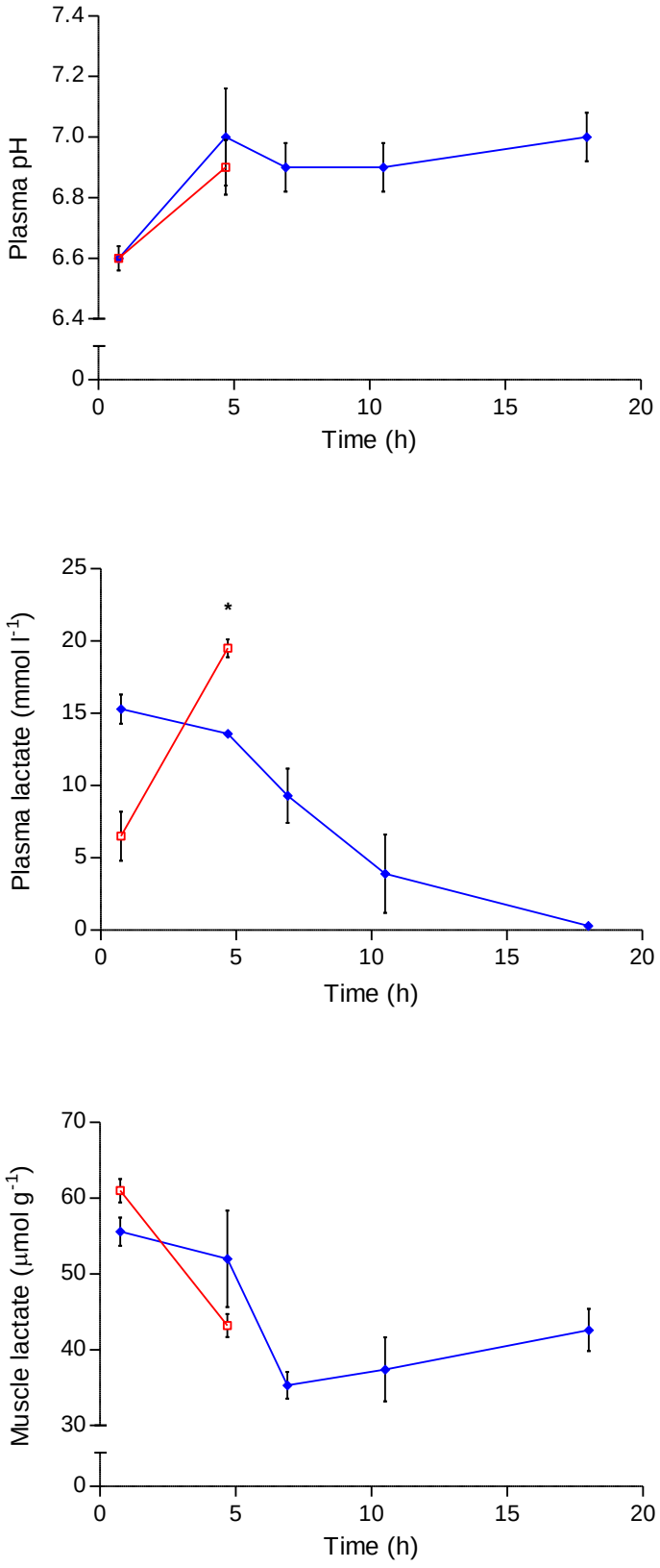


Figure 6.6. Changes in plasma pH and plasma and muscle lactate concentrations in snapper that suffered post-capture mortality (□), and those that survived until their allocated sampling time (◆).

6.4 Discussion

Characterising the physiological condition of fish caught by commercial fishing methods is important in understanding the nature and magnitude of the stresses experienced during capture and handling, which may have significant implications for the fitness and survival of juvenile and non-target fish that are discarded as bycatch, or for the quality of the harvested catch (Huss, 1995; Bjordal, 2002; Rotabakk et al., 2011). To our knowledge, this is the first study to investigate in detail the physiological response of snapper to capture by commercial trawl gears, and of their potential for metabolic recovery.

6.4.1 Limitations and caveats

The conclusions drawn by the present study are associated with a number of limitations or caveats, which have been acknowledged ahead of the following discussion since they may be important to the interpretation of the data. Some of these limitations are inherent within trawl-based field studies; for example, significant financial and logistical constraints associated with both the capture and retention of fish may restrict the scope and replication of a study (Davis, 2002; Broadhurst et al., 2006), and in the present case, limited the resolution of the physiological response to capture by restricting the number of sampling times that could be examined. The variety of potential capture stressors and the inability to control conditions *in situ* may result in significant variation in the condition of fish post-capture, both within and between capture events (Davis, 2002). Finally, field studies are also associated with a lack of suitable controls, in particular, of fish representing a rested or unstressed condition by which to measure the magnitude of any stress response, or with which to gauge the potentially confounding effects of containment-induced stress during the retention of fish post-capture (Davis, 2002; Broadhurst et al., 2006).

With regards to the present study, only fish that were alive and in comparatively good physical condition at the time of landing were set aside for subsequent sampling. The physiological response described may therefore be considered somewhat of a best-case scenario, and may underestimate the magnitude of the disturbances and/or mortality of fish in poorer condition at landing; this is certainly implied by the apparent association between lethargy and mortality in fish landed during the first trawl. Further, the response to capture

was determined only for relatively large fish; at a mean fork length of 38 cm, these fish were well in excess of the minimum legal size (MLS) for commercial capture of 25 cm, and were therefore not representative of the fish that would be discarded as bycatch. Snapper exceeding the MLS but caught in the absence of appropriate quota (for example, as bycatch or in excess of one's existing allowance) would still be retained; the QMS encourages the landing of all fish of legal size – with appropriate penalties in the form of deemed values – to allow the appropriate documentation of the catch to occur (Peacey, 2003). Size may play a significant role in the response to capture, with smaller fish often showing greater sensitivity to capture stressors and resultant mortality (Nielsen, 1989; Suuronen et al., 1995, 1996*a*, 1996*b*; Sangster, 1996; Davis and Olla, 2002; Davis and Parker, 2004; Stewart, 2008). As swimming capacity (as a measure of absolute velocity) scales positively with size (Bainbridge, 1960, 1962; Brett, 1965; Fry and Cox, 1970; Beamish, 1978; Kieffer, 2000), smaller fish are more likely to experience fatigue at a given tow speed than are larger fish, with the failure to maintain controlled movement, or indeed to maintain station at all, resulting in the increased incidence of abrasion and impact injuries, and/or asphyxia as fish become pinned against the net or the accumulating catch (Davis, 2002; Broadhurst et al., 2006). Smaller fish may also be more sensitive to thermal stress, given the more rapid rate at which their core body temperature changes (Davis et al., 2001; Davis and Olla, 2001, 2002), and to crush injury (Davis, 2002) and barotrauma (Stewart, 2008) during haulback.

It should also be noted that although tissue samples were placed on ice and within a freezer immediately following excision, the unfortunate inability to utilise liquid nitrogen during the present study may lend some uncertainty as to the absolute values of some tissue metabolites. For example, some residual metabolism may have occurred during the period over which the muscle cooled, leading to elevated lactate and reduced glycogen levels. However, measurements such as WM pH, which was determined at the time of sampling, or plasma pH, lactate and cortisol, which are more stable, should still be accurate measures of the post-mortem condition.

6.4.2 Physiological condition at landing and potential for metabolic recovery

In the absence of control samples detailing the metabolic condition of unstressed, wild snapper it is difficult to determine the exact magnitude or duration of capture-induced

perturbations; nevertheless, using metabolite concentrations and general recovery profiles established within the literature and preceding chapter as points of reference, it was possible to infer the physiological changes that occurred as a result of capture. Snapper exhibited typical manifestations of physical exhaustion and stress, with evidence of significant plasma and tissue acidoses, depletion of glycogen within the WM and a concomitant accumulation of lactate, and the release of cortisol into the bloodstream. The mechanisms involved in these changes and the way in which they relate to snapper have been discussed previously (see Chapter 5), and will therefore not be revisited here; rather, the following discussion will focus on the physiological condition of trawl-caught fish, the recovery from capture-induced perturbations, and the potential correlation of physiological measurements with mortality.

Muscle lactate concentrations in the hour post-capture were amongst the highest reported for physically exhausted fish, with few studies – laboratory- or field-based – reporting concentrations in excess of 40 μmol g^{-1}. Lactate levels of ~45 μmol g^{-1} were measured in snapper caught by longline (Lowe et al., 1993), and concentrations exceeding these levels reported for coho salmon (*Oncorhynchus kisutch*) caught by trolling, gillnetting and seining (Farrell et al., 2000; Farrell et al., 2001*a*, 2001*b*), trawl-caught Atlantic cod (*Gadus morhua*; Fraser et al., 1965), and for rainbow trout (*Oncorhynchus mykiss*) chased to exhaustion within the laboratory (Stevens and Black, 1966). Consistent with the substantial accumulation of lactate is the near total depletion of glycogen from within the WM. Several laboratory-based studies have reported that exhaustive exercise resulted in the depletion of 90% of the WM glycogen reserves (Pagnotta and Milligan, 1991; Wang et al., 1994*a*; Richards et al., 2002*a*). Although muscle glycogen concentration may vary with a range of factors, including temperature and nutritional status, if the glycogen levels of rested snapper were presumed to be similar to those previously reported for snapper (~20 μmol g^{-1}; Cook and Herbert, 2012; Tuckey et al., 2012), a post-capture concentration of 0.6 μmol g^{-1} represents a reduction of approximately 95%. Glycogen was similarly depleted in trawl-caught Atlantic cod, where rested levels estimated at 30 μmol g^{-1} were reduced to <1 μmol g^{-1} (Fraser et al., 1965). Glycogen levels of ~1 μmol g^{-1} have also been documented in trawl-caught haddock (*Melanogrammus* aeglefinus; Fraser et al., 1965), and gillnet- and troll-caught coho salmon (Farrell et al., 2000). Although it is possible that lactate concentration may be somewhat elevated due to methodological constraints, the low intracellular pH – obtained at the time of

sampling – is further consistent with the extent of glycogen depletion and lactate accumulation observed within the WM, and is similar to the pH recorded in the WM of trawl-caught Altantic cod (Rotabakk et al., 2011). Several laboratory-based studies have reported WM pH of 6.6-6.7 in rainbow trout subjected to exhaustive exercise (Milligan and Wood, 1986; Schulte et al., 1992; Wang et al., 1994*a*; van Ginneken et al., 2008).

Considering the extent of lactate production within the WM, the elevation of plasma lactate is relatively modest: commercially harvested coho salmon exhibiting muscle lactate loads similar to those observed in the present study show peak plasma lactate levels of 20 to 30 mmol l^{-1}, while concentrations in excess of 20 mmol l^{-1} have been reported elsewhere for troll-caught coho and Chinook salmon (*Oncorhynchus tshawytscha*; Parker and Black, 1959; Parker et al., 1959; Ellis, 1964). These differences may suggest species-specific differences in lactate dynamics, for example in membrane permeability or capacity for lactate reuptake, and hence the net loss of lactate to the plasma. Alternatively, the discrepancy may reflect continues aerobic metabolism within the WM during the time requires for the tissue to freeze. The differences may also reflect the latency associated with changes in plasma lactate concentration, since peak levels are typically observed 1 to 2 hours following a laboratory-based exhaustive event (Milligan and Wood, 1986; Pagnotta et al., 1991; Wang et al., 1994*a*; Milligan et al., 2000), with some field studies reporting peak lactate concentrations as late as 4 hours post-capture (Parker and Black, 1959; Beamish, 1966); the lactate load may therefore have peaked during the interval between the first and second sampling times. The same consideration may apply to the changes observed in plasma cortisol concentration; cortisol levels were elevated at landing in comparison to values previously reported for rested snapper (<10 ng ml^{-1}; Pankhurst and Sharples, 1992; Lowe et al., 1993), but were considerably less than have been observed for other species in response to capture, confinement, handing and vigorous swimming activity (often up to 400-500 ng ml^{-1}; see Barton and Iwama (1991) for a compendium). Further, the absence of a characteristic peak in cortisol, often observed 1 to 2 hours following exposure to a significant stressor (Pickering et al, 1982; Milligan et al., 2000; Milligan, 2003), suggests that peak cortisol levels may have occurred between the two sampling times.

Metabolic recovery was evident by the time at which the second set of samples were taken at 4.7 hours post-capture, with muscle and plasma pH increasing, and indications of the clearance of lactate from the muscle. Assuming the reduction in plasma pH attributed to the

use of the K_2EDTA-coated vacutainers was consistent at 0.3 pH units across all samples obtained from trawl-caught fish, plasma pH returned to levels consistent with those reported for rested snapper within the laboratory within 4.7 hours, followed by the clearance of plasma and liver lactate loads between 10 and 18 hours post-capture. Muscle pH increased progressively over the 18 hours during which physiological condition was assessed; although comparative values for laboratory-exercised snapper are not available, Tuckey et al. (2012) reported WM pH of 7.6 for rested snapper, hence it might be expected that intracellular acid-base balance is restored ~18 hours post-capture, and pH would stabilise at subsequent time-points. Although significant clearance of muscle lactate is evident over the first 6.9 hours post-capture, the reasons for the increase in lactate at 18 hours post-capture is unclear, since it is not associated with a reduction in either pH or glycogen. Finally, following their initial liberation in response to capture stress (van den Boon, 1991; Mommsen, 1999), plasma and muscle glucose remained elevated for an extended period, consistent with the relative glucose intolerance typical of fish (Moon, 2001), before returning to levels consistent with those of rested fish around 18 hours post-capture.

However, not all physiological parameters exhibited signs of recovery within the 18 hour period during which the physiological condition was assessed. Muscle glycogen remained depressed throughout the recovery period, as did liver glucose and glycogen concentrations, and plasma cortisol levels remained chronically elevated. The elevation of cortisol following strenuous activity has been implicated in the delay of metabolic recovery (Pagnotta et al., 1994; Eros and Milligan, 1996; Milligan et al., 2000; Milligan, 2003). In particular, cortisol is thought to inhibit glycogen restitution through the promotion of glycogen phosphorylase activity and glycogenolysis (Milligan, 2003; Frolow and Milligan, 2004). The decline in liver glycogen post-capture is also likely a function of the activity of cortisol; the liver represents an important energy reserve, and the liberation of hepatic glycogen has previously been documented in fish in response to stress (Paxton et al., 1984; Vijayan et al., 1990; Vijayan and Moon, 1992) or the administration of exogenous cortisol (Barton et al., 1987; Vijayan et al., 1991), although the response may be species-specific and depend on the magnitude of the stress and/or energetic state of the animal (Vijayan and Moon, 1992). The depletion of hepatic glycogen was documented in trawl-caught herring (*Clupea harengus*), and remained depressed over the following 5 days, despite the availability of food (Suuronen et al., 1996*a*). Potential differences in the nutritional state of wild and captive snapper may explain the

pronounced differences in the liver glycogen reserves observed in the two groups of fish (Heinimaa, 2003). Whether the continued elevation of cortisol represents simply a protracted recovery as consequence of the magnitude of the stress experienced, or indicates an additional stress response associated with confinement within the holding tanks remains unclear, since it is difficult to separate the two possibilities in the absence of adequate controls. Chronic stress associated with the retention of fish post-capture has previously been implicated in the impairment of recovery from capture (Ellis, 1964; Parker et al., 2003), as the conditions in which fish are held are often dissimilar to those of their natural environment, differing in terms of light intensity, temperature, pressure and density of individuals (Broadhurst et al., 2006). Periodic disturbance associated with sampling may also have contributed to a possible holding stress.

6.4.3 Comparison of the physiological condition and recovery of snapper following laboratory-based exercise and commercial capture

In general, the physiological perturbations experienced by snapper during capture by trawl fishing were qualitatively similar to those experienced by fish subjected to exhaustive exercise within the laboratory, with the severity of the response, as might have been expected, being greater in trawl-caught fish. In particular, trawl-caught snapper developed a larger lactate load than did laboratory-exercised fish, evidenced by the elevated concentrations within the WM and liver, and a protracted recovery of the plasma lactate load. Consistent with the greater reliance on anaerobic energy production was a more severe acidification of the plasma (the effects of the K_2EDTA vacutainers being accounted for). In contrast with the rapid recovery of exercise-induced perturbations exhibited within the laboratory, physiological recovery of trawl-caught fish was prolonged, with some metabolites demonstrating residual disturbance and others no indications of recovery following the 18 hour period for which physiological condition was assessed. In particular, plasma cortisol remained elevated, although whether this reflects the magnitude of the stress experienced during capture, or limitations of the holding conditions remains unclear; the impairment of glycogen restitution within the WM and liver may be a function of this extenuating stress.

The direct comparison of the metabolic perturbations that occur in response to capture in trawl-caught with those of laboratory-exercised fish should be with due caution, considering

the uncertainties as to the precise nature of the lactate load developed in the latter (as discussed in Chapter 5), and further, distinct differences in the size of snapper, and to a lesser degree, in temperature between the two studies, both of which have been shown to influence the magnitude of the lactate load developed in response to exhaustive exercise and stress (Goolish, 1989; Fergusson et al., 1993; Kieffer et al., 1996; Jain and Farrell, 2003; MacNutt et al., 2004). Nonetheless, the depletion of hepatic glycogen and the observation of mortality in trawl-caught fish alone attest to the severity of the stress experienced by snapper during capture, and the greater impacts of these stressors on the physiological condition of snapper post-capture.

6.4.4 Delayed mortality and its correlation with physiological indicators

The accurate estimation of mortality rates in fish discarded as bycatch during commercial fishing is essential to effective stock management (Davis, 2002; Diamond and Campbell, 2009; Frick et al., 2010). In the present study, snapper suffered moderate rates of mortality as a result of capture in commercial trawl gears. While logistical constraints associated with the size of the trawl and the operation of several concurrent experiments precluded the determination of immediate mortality rates, the majority of the catch was landed alive. Of the fish that were alive at landing and subsequently retained in holding tanks for the assessment of post-capture condition, 14% suffered delayed mortality, all of which was observed within the first 5 hours post-capture. Significant mortality has previously been reported in snapper captured by various commercial fishing methods. Juvenile snapper caught as bycatch in a trawl-based prawn fishery suffered mortality rates of 35%, increasing to 100% as the duration for which fish were exposed to the air during sorting increased from 5 to 30 minutes (Sumpton and Jackson, 2005). In snapper caught in commercial traps, mortality was strongly correlated with capture depth, increasing from <2% at <30 m to 55% at 44-59 m (Stewart, 2008), highlighting the potential for barotrauma to cause significant mortality in snapper. Smaller snapper were found to be especially vulnerable, with a higher incidence of mortality than larger fish (Stewart, 2008).

It is likely that the present estimate of discard mortality in trawl-caught snapper underestimates the mortality rates that may be observed in the commercial setting. As discussed above, no attempt was made to account for mortality occurring during or immediately following capture. Further, delayed mortality was determined in fish that were in comparatively good physical condition at landing, and were in excess of the minimum legal size restrictions, and would therefore almost certainly be retained as catch; smaller fish, with their tendency to higher mortality rates (Suuronen et al., 1995, 1996*a*, 1996*b*; Sangster, 1996; Stewart, 2008), would likely constitute the majority of any discarded catch. In addition, mortality may be significant over a period of days to weeks following the capture and discard event (Oddson et al., 1994; Turunen et al., 1994; Olla et al., 1997, 1998; Davis and Olla, 2001, 2002; Parker et al., 2003; Davis, 2005), as consequences such as the infection of wounds begin to manifest (Broadhurst et al., 2006). Logistical constraints severely limited the duration for which fish could be retained post-capture, and as such, mortality that may have otherwise been observed during an extended monitoring period, may not have been accounted for. This unaccounted mortality may have been compounded by the use of terminal sampling methods during the determination of physiological status, which while appropriate in terms of the primary objective of the study being to characterise the physiological perturbations induced by capture and their potential for recovery, progressively reduced the sample size available for the monitoring of mortality, and may have masked mortality that may have otherwise been observed.

The reason(s) for the differences in the degree of lethargy shown by fish at landing and of subsequent mortality between the two trawls remain unclear. The gear configurations, tow speed, environmental temperature and the size of fish assessed were consistent between the tows; however, the first trawl was approximately 20% longer in duration and had a catch size approximately 15% larger than that of the second trawl. Increased tow duration has been correlated with mortality (Oddson et al., 1994; Hatulla et al., 1995; Olla et al., 1997; Frick et al., 2010; Olsen et al., 2013) and behavioural impairment of survivors (Olla et al., 1997), as the effects of fatigue are amplified and the risks of net entrainment, crush injury and/or asphyxia are increased. The effects of catch size on the condition and survival of fish has received considerably less attention, but has also been associated with higher mortality (Turunen et al., 1994; Suuronen et al., 2005). It is assumed that increased catch size increases the likelihood of injury associated with collision with other fish or with the net, and may

magnify crush injury within the codend or during haulback (Oddson et al., 1994; Suuronen, 2005). Crowding stress associated with catch size significantly influenced mortality in mackerel during simulated purse seine capture (Lockwood et al., 1983). Larger catch size is also associated with increased sorting time, which may confer higher rates of mortality (Sumpton and Jackson, 2005). Although the apparent association between tow duration and/or catch size and mortality observed in the present study is based on only two trawl events, and notwithstanding the high levels of between-catch variation to which field studies are prone (Beamish, 1966; Davis, 2002), these observations suggest that the role of tow duration and catch size in determining the response to capture warrants further investigation.

Given the potential for mortality to occur over an extended period post-capture and the logistical constraints involved in the retention of fish over a prolonged period, a more efficient means of estimating discard mortality may be prediction based on the physiological condition of fish at landing or shortly thereafter (Davis and Ottmar, 2006). Of the physiological parameters measured in the present study, only plasma lactate displayed any differences in post-capture dynamics between fish that suffered mortality and those that did not, showing significant increases in concentration in fish that died, in contrast to the recovery evident in surviving fish. Plasma lactate has previously been shown to correlate with mortality in Pacific halibut (*Hippoglossus stenolepis*), Chinook salmon, sablefish (*Anoplopoma fimbria*), red snapper (*Lutjanus campechanus*) and haddock (Parker and Black, 1959; Beamish, 1966; Olla et al., 1998; Davis and Schreck, 2005; Diamond and Campbell, 2009); however, the relationship is inconsistent, with a lack of association demonstrated in other studies (Oddson et al., 1994; Davis et al., 2001). Plasma lactate has also been correlated with the impairment of swimming capacity, with the critical swimming performance of trout and salmon exhibiting high concentrations of plasma lactate reduced during repeat swimming challenges (Farrell et al., 1998; Jain et al., 1998; Jain and Farrell, 2003; MacNutt et al., 2004). The impairment of swimming performance may facilitate indirect mortality or reduced fitness through compromised predator detection and evasion, foraging behaviour or migration of anadromous fish (Olla et al., 1997; Ryer, 2004). In the present study, since the lactate load within the WM did not differ between surviving and non-surviving fish, differences in plasma lactate were not reflective of differences in lactate production, but may indicate altered lactate dynamics, resulting from some compromised physiological state. The depletion of high-energy phosphates has previously been implicated in mortality resulting from exhaustive

exercise (van Ginneken et al., 2008) and exposure to hypoxia (van Ginneken et al., 1995). Impairment of essential energy-consuming processes such as transmembrane ion flux may lead to irreversible changes in cell membrane potential, resulting in cell death (Boutilier, 2001); the deterioration of ion flux could explain the sudden increase in plasma lactate in non-surviving fish, since lactate is actively retained within the WM (Milligan and Girard, 1993; Laberee and Milligan, 1999; Sharpe and Milligan, 2003). Whether these changes in plasma lactate are a true indicator of mortality or occurred post-mortem remains to be determined. The absence of any association between mortality and plasma cortisol or glucose is consistent with the lack of concordance generally observed, regardless of species or fishing method (Oddsson et al., 1994; Davis et al., 2001; Davis, 2002; Parker et al., 2003; Davis and Schreck, 2005).

The inconsistencies in the relationship between plasma constituents and mortality between studies reflects the diversity of stress responses observed in relation to differences in species, size, capture and handling methods and environmental conditions, and further, that mortality may result from one or a combination of several potential stressors, not all of which may manifest as changes in plasma constituents. For example, although plasma lactate indicates severe physical exertion, which may in itself result in mortality (Wood et al., 1983; van Ginneken et al., 2008), it may not reflect injury such as barotrauma, scale loss or the infection of abrasions, all of which may induce mortality as a single stressor (Davis and Ottmar, 2006; Stewart, 2008; Olsen et al., 2012). Variation in the magnitude of the physiological perturbations resulting from stress may vary with size (Ferguson et al., 1993; Kieffer et al., 1996) and temperature (Jain and Farrell, 2003; MacNutt et al., 2004), which may introduce further difficulties in establishing meaningful indicator or threshold values. This is not to suggest the measurement of plasma lactate is of little value; plasma lactate is often used as a proxy for metabolic effort and may therefore prove useful as an indicator of metabolic condition and hence potential quality of the fillet. In terms of mortality, it may be of use as part of a more integrative condition index; Davis (2005) suggested that a comprehensive index for the prediction of mortality should incorporate indicators of a range of potential sources of mortality, including physical injury (i.e. symptoms of barotrauma or abrasion), physiological stress (i.e. plasma cortisol or lactate) and behaviour (i.e. the ability to maintain orientation or the vigour with which fish struggle). Condition indices incorporating multiple potential stressors have been correlated

with mortality in sablefish (Davis, 2005) and red snapper (Diamond and Campbell, 2009). Alternatively, the impairment of reflex behaviours, such as the startle response, reflex closure of the mouth or opercula, or gag and vestibular-ocular reflexes may provide an indication of the overall physiological condition of the animal, and its ability to function at the most basic physiological level. Reflex impairment indices have been used successfully in the prediction of mortality in a number of species, including sablefish, walleye pollock (*Theogramma chalcogramma*), northern rock sole (*Lepidosetta polyxystra*), Pacific halibut and coho salmon (Davis and Parker, 2004; Davis, 2005; Davis and Ottmar, 2006; Davis, 2007). Investigation of reflex impairments or other condition indices in snapper may therefore be of value with regards to creating a more robust index for the prediction of tissue quality and/or mortality.

6.5 Conclusions

Snapper caught by commercial trawl fishing experienced significant stress and physical fatigue, exhibiting a near-total depletion of muscle glycogen and associated accumulation of lactate and acidosis of the muscle and plasma. The physiological response was qualitatively similar to that induced by a laboratory-based exhaustive exercise test; however, perturbations observed following capture were of a greater magnitude and demonstrated a protracted recovery. In particular, although there was evidence of the correction of the lactacidosis within the muscle and plasma, cortisol levels remained elevated, and muscle and liver glycogen levels depressed, indicating a persistent stress response, possibly as a result of confinement in on-board holding tanks. In trawl-caught snapper retained as catch, the energy-deplete state of the muscle at landing presumably accelerates the onset of autolytic reactions post-mortem, with implications for the quality of the fillet. In fish discarded as bycatch, delayed mortality may be significant – the observed rates of 14% likely underestimate those observed in a commercial setting. Further, the implications of chronic stress and delayed metabolic recovery for indirect mortality and/or fitness remain unknown. As the response to capture may vary in relation to numerous biological, environmental and operational conditions, future work should seek to determine the effects of these on the physiological condition and survival of snapper; the

present study suggests that the effects of trawl time warrants further investigation, while the effects of fish size, temperature, tow speed and handling processes are also likely to be important in characterising the response to capture.

CHAPTER 7

Tolerance of hypoxia and its effects on swimming performance and recovery from exercise

7.1 Introduction

7.1.1 Defining hypoxia

The reduction of environmental oxygen, known as hypoxia, is a naturally occurring phenomenon of aquatic environments. The low oxygen capacitance of water renders it vulnerable to depletion by respiring organisms, in particular where geographic boundaries (e.g. estuaries) or stratification (thermo- and haloclines) may limit mixing with oxygen-laden waters (Diaz and Breitburg, 2009; Domenici et al., 2013). Further, nutrient loading and eutrophication of coastal marine environments are associated with an increase in the prevalence and intensity of hypoxic events, such that dissolved oxygen represents, by far, the most changed of the environmental parameters associated with marine environments over the last 50 years (Diaz, 2001; Vaaquer-Sunyer and Duarte, 2008; Diaz and Brietburg, 2009). In addition to naturally occurring hypoxia, oxygen limitation is frequently observed within aquaculture ventures, as a consequence of high stocking densities (Fitzgibbon et al., 2007; Wu et al., 2009).

Quantitative definitions of the degree of oxygen depletion that constitutes hypoxia are variable or often totally absent. For example, hypoxia is commonly defined by regulatory organisations as dissolved oxygen levels less than 2-3 and 5-6 mg l^{-1} for marine and freshwater environments, respectively (Wu, 2002; Farrell and Richards, 2009); however, the extensive literature review by Vaquer-Sunyer and Duarte (2008) demonstrated that the sub-lethal and lethal oxygen concentrations of a range of marine taxa may be considerably higher. Further, since species vary significantly with respect to both their requirements for oxygen

and their ability to compensate for changes in environmental oxygen availability, physiologists typically define hypoxia as the level of oxygen depletion at which physiological function becomes impaired (Chabot and Claireaux, 2008; Farrell and Richards, 2009). Indeed, this definition will apply throughout the current study.

7.1.2 The metabolic challenge of hypoxia

Oxygen is essential to the production of ATP by oxidative phosphorylation, and hence environmental oxygen availability represents a powerful limiting factor governing the metabolic performance of aquatic organisms (Fry, 1947; Claireaux et al., 2000; Chabot and Claireaux, 2008). The extraction of oxygen from the environment and its subsequent delivery to the mitochondria occurs via a series of diffusive cascades, determined by the partial pressure gradients across several levels of biological organisation (Hughes, 1973, 1981). As ambient oxygen tensions are reduced, the gradient for the diffusion of oxygen across the gill lamellae is reduced; hypoxia may therefore limit the capacity for oxygen uptake, with consequences for aerobic metabolism.

The constraints imposed on metabolism by hypoxia are illustrated by the "scope for activity" framework developed by Fry (1947; Fig. 1). The majority of fish species are oxygen-regulators, and are able to maintain $MO_{2\ standard}$ across a range of ambient oxygen tensions. As PO_2 declines, adjustments in cardiorespiratory function act to maintain oxygen uptake and preserve arterial blood oxygen content, such that basal metabolic requirements continue to be met (Gamperl and Driedzic, 2009; Perry et al., 2009). These adjustments may include an increase in ventilatory frequency and/or stroke volume (Hughes, 1973; Randall, 1982; Perry et al., 2009), gill perfusion and lamellar recruitment (Booth, 1979), alterations in blood pressure and blood flow distribution (Gamperl and Driedzic, 2009; Janssen et al., 2010) and erythrocyte recruitment (Yamamoto et al., 1983; Cook and Herbert, 2012). These changes are initiated following the detection of reduced ambient and/or arterial PO_2 by oxygen-sensitive neuroepithelial cells (NEC) in the gill epithelium and are coordinated by the autonomic nervous system (Taylor et al., 1999; Burleson, 2009; Perry et al., 2009). Ultimately, the capacity of the respiratory and circulatory systems to further compensate for the continued decline in PO_2 is exceeded, and oxygen uptake becomes insufficient to sustain

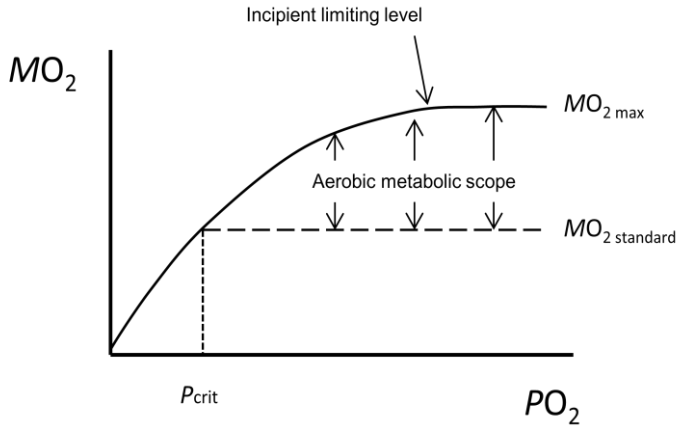


Figure 7.1. Conceptual overview of the metabolic response of fish to hypoxia. As ambient PO_2 is reduced below the incipient limiting level, $MO_{2\ max}$ and thus aerobic scope are reduced. The relation between $MO_{2\ max}$ and PO_2 is described by the limiting oxygen concentration (LOC) curve. At P_{crit}, $MO_{2\ max}$ is equal to $MO_{2\ standard}$, such that there is no scope for activity. At PO_2 below P_{crit}, oxygen uptake is insufficient to sustain $MO_{2\ standard}$, and the animal transitions to an oxygen-conforming state. Modified from Fry (1947).

$MO_{2\ standard}$. The PO_2 at which $MO_{2\ standard}$ can no longer be maintained is described by the critical oxygen tension, P_{crit} (Pörtner and Grieshaber, 1993; Farrell and Richards, 2009; Richards, 2009). At PO_2 below P_{crit}, the animal transitions to an oxygen-conforming state, with oxygen consumption directly dependent on ambient PO_2. The resulting limitation on aerobic ATP production therefore requires the supplementary recruitment of anaerobic glycolysis to meet metabolic demand (Pörtner and Grieshaber, 1993; Boutilier, 2001; Farrell and Richards, 2009; Richards, 2009). For fish that are unable to relocate from such oxygen-limited environments, survival is time-limited. Cessation of anaerobic ATP production due to the depletion of glycogen and/or end-product (H^+, P_i) inhibition results in the failure of ionoregulatory mechanisms, a loss of cellular homeostasis and necrotic cell death (Boutilier, 2001; Vornanen et al., 2009). The high intrinsic rates of ATP consumption by excitable cells, in particular, cardiac and brain tissues, render them especially sensitive to a reduced oxygen supply; brain dysfunction and cardiac failure are primary causes of hypoxia-induced mortality (Boutilier, 2001; Richards, 2009; Vornanen et al., 2009).

During periods of maximum oxygen uptake, the cardiovascular and respiratory systems function at their maximum capacity, thus fish have little scope by which to compensate for the effects of reduced oxygen availability on oxygen uptake; $M\mathrm{O}_{2\ \mathrm{max}}$ is therefore sensitive to even small fluctuations in ambient oxygen tension (Fry, 1947; Fitzgibbon et al., 2007; Farrell and Richards, 2009). By extension, aerobic metabolic scope is increasingly reduced with the progressive reduction of ambient $P\mathrm{O}_2$. The $P\mathrm{O}_2$ at which limitations in $M\mathrm{O}_{2\ \mathrm{max}}$ and scope become evident is defined by Fry (1947) as the "incipient limiting level" (Fig. 7.1). Thus, hypoxia may have not only profound implications for immediate survival, but at sub-lethal levels may reduce the aerobic energy budget, with depressive effects on swimming performance, digestive function, growth, reproductive development and success, and immune function (Wu, 2002, 2009; Chabot and Claireaux, 2008; Chapman and McKenzie, 2009; Diaz and Breitburg, 2009; Wang et al. 2009).

7.1.3 The importance of hypoxia in fisheries

The physiological effects of hypoxia on fish are of increasing interest to the fisheries sector, in particular with respect to aquaculture, where hypoxia is frequently problematic (Fitzgibbon et al., 2007; Wang et al., 2009). Since metabolic scope determines the capacity for activities such as growth, reproductive development and immune function, maintaining culture conditions that ensure that fish may express their maximum scope will maximise potential productivity (Fitzgibbon et al., 2007; Chabot and Claireaux, 2008). In contrast, the role of hypoxia in capture fisheries has received little attention. Davis (2002) suggests that fish may experience a localised hypoxia within the codend of trawl gears, given the accumulation of maximally-respiring fish; however, the possible magnitude of oxygen depletion and its impact on the swimming performance and behaviour of fish within the net is unknown.

In addition, hypoxia may have important implications for recovery and survival post-capture, since reduced ambient oxygen tensions may constrain the excess post-exercise oxygen consumption (EPOC) associated with the correction of exercise- and stress-induced perturbations in homeostasis (Svendsen et al., 2012; Domenici et al., 2013). Hypoxia may therefore be an important consideration regarding the retention of fish within on-board

holding facilities, for example, in which fish are retained for subsequent provision to live-fish markets or to permit recovery of juvenile and non-target fish prior to discard (e.g. the Fraser box; Farrell et al., 2001*a*).

7.1.4 Objectives

A limited literature exists describing the hypoxia tolerance of the snapper, *Pagrus auratus* (Cook et al., 2011, 2013; Patel, 2011; Cook and Herbert, 2012). Cook and colleagues have described snapper as being moderately tolerant of hypoxia, with a P_{crit} between 40 and 54 mmHg, depending on size and temperature, and avoidance behaviours invoked at ambient PO_2 of approximately 22-24 mmHg. The aims of the current chapter were to further examine the hypoxia tolerance of snapper, including the corroboration of P_{crit} data, and the measurement of terminal PO_2, P_{term}, as an indicative measure of the PO_2 at which equilibrium is lost and asphyxia is imminent. The adjustments in cardiovascular function that occur in snapper in response to hypoxia were also investigated. Finally, the influence of hypoxia on the exercise physiology of snapper was explored, by characterising effects of hypoxia on critical swimming performance, and further, assessing the recovery of $MO_{2\ post\text{-}exercise}$ in fish exposed to different PO_2.

7.2 Materials and Methods

7.2.1 Experimental set-up

For respirometry-based elements of this chapter, the automated respirometry system was used as described in Section 2.4. Fish were lightly anaesthetised in MS-222, weighed and measured, and placed into the respirometer, with a minimum 18-hour recovery period observed prior to the start of any experimental work. Oxygen electrodes were calibrated 1 hour prior to commencing the recording of MO_2, with care taken not to disturb the fish.

Upper and lower PO_2 thresholds of 140 and 120 mmHg respectively were maintained, unless otherwise stated.

All experimental work was carried out at 18 ^{o}C.

7.2.2 Determination of critical and terminal PO_2

Snapper were instilled within respirometers a minimum of 18 hours prior to the start of experimental work, as described. The experiment commenced with the closure of the solenoid and sealing of the respirometer; PO_2 was then reduced by allowing the fish to consume the available oxygen from within the respirometer. MO_2 was determined at 10 mmHg increments by calculating the rate of oxygen depletion (i.e. the slope of the trace) 2 mmHg either side of the given PO_2. For example, to calculate MO_2 at a PO_2 of 100 mmHg, the rate of oxygen depletion from 102 to 98 mmHg was determined. The experiment was terminated at the point where fish exhibited signs of distress, characterised by high-intensity, escape-type swimming activity, and which preliminary experiments indicated were rapidly followed by a loss of equilibrium. At this point, the respirometer was flushed with aerated water. The PO_2 at which the experiment was terminated was recorded as the terminal PO_2, P_{term}. The time required for the fish to deplete oxygen tensions to this point varied, depending on the size of the fish and volume of respirometer used (30-60 minutes for 20 g fish, 2-2.5 hours for 150 g fish and 1.0-1.5 hours for 230 g fish; see below).

For the calculation of P_{crit}, $MO_{2\ routine}$ was first determined by taking the mean rate of oxygen consumption for PO_2 above 120 mmHg, consistent with the normoxic, resting conditions employed throughout this thesis. P_{crit} was subsequently determined using a method similar to that described by Schurmann and Steffensen (1997) and Cook and Herbert (2012). A repeated-measures ANOVA with Bonferroni post-hoc comparison was used to deduce the PO_2 increments at which MO_2 was significantly different to $MO_{2\ routine}$; a linear regression was fitted through these points, and constrained through the origin. $MO_{2\ routine}$ was then extrapolated across the entire PO_2 range, and the intercept of $MO_{2\ routine}$ with the linear regression used to define P_{crit}.

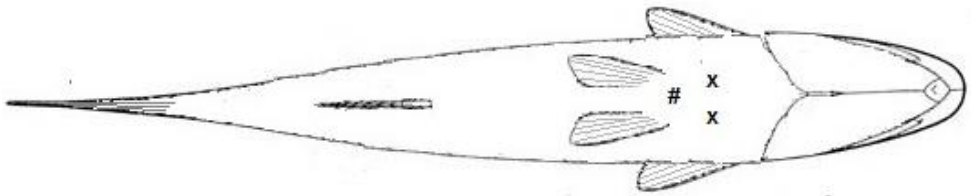
To investigate whether hypoxia tolerance may be size-dependent, critical and terminal PO_2 were determined for 2 size classes of snapper, hereafter referred to as the 20 g (18.7 $\pm$ 1.2 g; n = 8) and 150 g (157.5 $\pm$ 6.8 g; n = 8) groups. Critical PO_2 was also determined for ~230 g snapper during the measurement of the cardiorespiratory response to hypoxia detailed in the following section.

To verify that the changes in MO_2 observed were the result of declining oxygen tensions rather than the accumulation of CO_2 within the respirometer, the above experiment was repeated with an additional group of fish (175.6 $\pm$ 10.4 g; n = 8). Nitrogen was slowly bubbled through the water reservoir, such that the respirometer was flushed with water of a progressively lower oxygen tension, while any CO_2 present was flushed out. When the PO_2 within the respirometer reached approximately 60 mmHg, the respirometer was sealed, permitting the determination of MO_2. As above, the experiment was terminated when fish exhibited signs of distress, at which point the respirometer was flushed with aerated water.

7.2.3 Cardiorespiratory response to hypoxia

To investigate the adjustments in cardiorespiratory function that occur in snapper during exposure to progressive hypoxia, heart and ventilation rates were determined by fitting electrocardiogram (ECG) electrodes as detailed below. The electrodes were connected to the Powerlab data acquisition system via a BioAmp amplifier (AD Instruments, Waverly, N.S.W., Australia). The incurrent ECG signal was then filtered into its cardiovascular and respiratory components using digital filters contained within the Chart v7.3 software (bandpass: 8-70 Hz and bandpass: 0.5-3 Hz, respectively).

Snapper (231 $\pm$ 12 g; n = 7) were anaesthetised in 80 mg l^{-1} MS-222, weighed and measured, then placed on a surgical table with their ventral surface exposed. Two lengths of Teflon-coated, stainless steel wire electrodes (7 stranded, 0.0010" bare, 0.0055" coated, A-M Systems, Inc.) were cut approximately 30 cm in length, and the top 2-3 mm of Teflon coating removed from the end of each wire. The exposed ends of the electrodes were inserted subcutaneously using a 23-gauge syringe needle; the sites of insertion were approximately 10 mm anterior of the pectoral fins and 5 mm apart (Fig. 7.2). The electrodes were secured with two sutures, the first approximately 5 mm posterior of the site of insertion, and the second to



.

Figure 7.2. Schematic representation of the ventral surface of snapper, depicting the sites of insertion of the two ECG electrodes (x) and of their suture (#) anterior to the pelvic fins.

the front spine of the dorsal fin. The surgery process took approximately 5 minutes; midway through this period, approximately 200 ml of aerated 80 mg l^{-1} MS-222 solution was injected slowly through the mouth to permit gas exchange at the gills, and to maintain anaesthesia. Following surgery, the fish was placed into the respirometer, which was flushed continuously with an aerated water supply. Snapper rapidly regained equilibrium when replaced into fresh seawater. Fish were then provided an 18 hour recovery period prior to the start of the experiment; to avoid the electrode wires tangling during this period, the trailing lengths were tucked into an Eppendorf tube.

Prior to the start of the experiment, the electrodes were carefully removed from the Eppendorf tube, and approximately 5 mm of Teflon coating was removed from the end of each electrode, which were then connected to the BioAmp. Whilst care was taken not to disturb the fish during this process, a half hour re-settling period was observed prior to the start of the experiment to compensate for any disturbance. This is less than for other studies throughout the thesis, however, was a constraint imposed by the propensity of the fish to gradually tangle the electrodes during their routine movement within the respirometer; the effective reduction in electrode length created tension on the electrodes and hence on the sutures, causing probable stress to the fish. We therefore sought to resolve the experiment prior to any stress becoming significant.

Resting heart and ventilation rates were recorded over a period of several minutes, after which the experiment commenced as the respirometer was sealed. The fish were allowed to

deplete the available oxygen to a tension of 30 mmHg over the course of 60 to 90 minutes. Heart and ventilation rates were recorded during the development of hypoxia and during immediate recovery on return to aerated water; thereafter, the extent of tangling of the electrodes and the resultant tension on the sutures caused agitation in some fish, requiring the termination of the experiment before a meaningful recovery period could be recorded.

7.2.4 Effect of hypoxia on swimming performance (U_{crit})

To understand how hypoxia might affect swimming performance, snapper (52.9 ± 1.8g) were randomly divided into groups of six; each group was subject to a U_{crit} protocol (as per Section 2.3) at one of seven different oxygen tensions: 40 mmHg, 50 mmHg, 60 mmHg, 80 mmHg, 100 mmHg, 120 mmHg and 150 mmHg. Nitrogen was bubbled through the water in the swimming flume to reduce PO_2 to the relevant level; all experiments were carried out at 18^{o}C. The fish was then placed into the flume and the protocol commenced, with a 30 minute habituation period allowing the fish to adjust to the new environment. For swimming trials performed at tensions of 40 and 50 mmHg, however, the habituation period was reduced to 15 minutes to avoid potential complications associated with prolonged exposure to hypoxia prior to starting the U_{crit} protocol.

7.2.5 Recovery from exhaustive exercise under hypoxic conditions

To investigate how oxygen limitation might affect the recovery of MO_2 following exhaustive exercise, fish were exposed to one of three PO_2 ranges during post-U_{crit} recovery: 60-80 mmHg, 90-110 mmHg or 120-140 mmHg (47.7 ± 23.7 g, 42.1 ± 1.9 g and 47.0 ± 2.7 g respectively; each n = 8). Routine metabolic rate was first determined as described above, after which the fish was transferred to the swimming flume and subjected to a U_{crit} protocol (as per Section 2.3); both $MO_{2\ routine}$ and U_{crit} were determined under normoxic conditions. During the interim period whilst the fish was in the flume, nitrogen was used to reduce the ambient PO_2 within the respirometer to the upper limit of the applicable range (i.e. 80, 110 or 140 mmHg). Following U_{crit}, the fish was returned to the respirometer, and over the

subsequent 24 hour recovery period for which $M\mathrm{O}_{2\ \text{post-exercise}}$ was determined, the Fast Response Output function was used to constrain $P\mathrm{O}_2$ within the required range.

7.2.6 Statistical analysis

P_{crit} was determined as described in Section 7.2.2. 95% confidence intervals are reported for the intercept of the regression of $P\mathrm{O}_2$-dependent $M\mathrm{O}_2$ and $M\mathrm{O}_{2\ \text{routine}}$. P_{term} was compared for 20 g and 150 g snapper using a Student's t-test of unpaired design.

Changes in heart and ventilation rates during the development of hypoxia were determined by way of repeated measures ANOVA, with Bonferroni post-hoc comparison used to deduce the oxygen tensions at which rates were significantly different to those of rested snapper at normoxic conditions.

The possibility of cardiorespiratory synchrony was investigated using the methods of Borch et al. (1993). The mean beat-to-beat interval (i.e. 1/frequency) was calculated for both heart and ventilation rates (designated Int(H) and Int(V), respectively). The frequency distribution of the ratio Int(H)/Int(V) was subsequently determined for each $P\mathrm{O}_2$ increment. Synchrony between the circulatory and ventilatory rhythms would be expected to manifest as nodes around the integers.

The effects of hypoxia on U_{crit} were determined by way of a one-way ANOVA with a Bonferroni post-hoc comparison to deduce the oxygen tensions at which U_{crit} was reduced relative to that of fish exercised at 150 mmHg.

Following U_{crit}, the time taken for $M\mathrm{O}_{2\ \text{post-exercise}}$ to return to pre-exercise rates was estimated by the pair-wise comparison of $M\mathrm{O}_{2\ \text{routine}}$ with $M\mathrm{O}_{2\ \text{post-exercise}}$ at subsequent sampling times, using a two-tailed Student's t-test of paired design (Scarabello et al., 1991, 1992). Excess post-exercise oxygen consumption (EPOC) was determined by calculating the area bound by the $M\mathrm{O}_{2\ \text{post-exercise}}$ curve and a y-value equal to $M\mathrm{O}_{2\ \text{routine}}$, between the time of exhaustion and the time at which $M\mathrm{O}_{2\ \text{post-exercise}}$ is equal to $M\mathrm{O}_{2\ \text{routine}}$ (Lee et al., 2003*a*, 2003*b*).

7.3 Results

7.3.1 Critical and terminal PO_2

Routine rates of oxygen consumption for 20, 150 and 230 g fish were 7.45 ± 0.3, 4.13 ± 0.25 and 4.20 ± 0.32 µmol g^{-1} h^{-1}, respectively – consistent with rates predicted by the allometric relations determined for snapper in Chapter 3. At high ambient oxygen tensions, snapper maintained a constant $MO_{2\ routine}$, despite reductions in PO_2 (Fig. 7.3). Below a critical PO_2, snapper of all sizes exhibited a linear decline in $MO_{2\ routine}$ with the continued reduction in PO_2. P_{crit} was subsequently resolved to 77 mmHg in 20 g fish (95% C.I. 71.9-82.1 mmHg), and 50 mmHg in both 150 and 230 g fish (95% C.I. 47.5-53.0 and 47.8-53.2 mmHg, respectively).

For a given sub-P_{crit} oxygen tension, the rate of oxygen consumption exhibited by snapper was independent of the method used to incite hypoxia; MO_2 was the same in snapper that had been allowed to slowly consume the available oxygen as it was for fish exposed to a rapid, nitrogen-induced hypoxia (Fig. 7.4).

Terminal PO_2 was significantly lower in 150 g fish than 20 g fish ($p = 0.0003$), at 22.2 ± 1.5 and 29.3 ± 1.1 mmHg respectively.

7.3.2 Cardiorespiratory response to hypoxia

Figure 7.5 shows a representative ECG recording, from which heart and ventilation rates were calculated. The resting heart rate of snapper under normoxic conditions was 74.1 ± 5.5 bpm and resting ventilation rate 75.6 ± 2.9 bpm.

As ambient oxygen tension was progressively reduced, snapper initially maintained a constant ventilatory frequency (V_f), with an increase in V_f evident as PO_2 was reduced below 100 mmHg. As PO_2 was further reduced, V_f continued to increase, reaching a maximum rate of 107.4 ± 3.3 bpm at 50 mmHg (Fig. 7.6). As PO_2 was reduced further still, fish began to exhibit signs of respiratory failure, with V_f at 30 mmHg significantly reduced compared with peak V_f ($p < 0.0001$). Although the ECG method used in the current study did not permit the quantification of ventilatory stroke volume, it was possible to visually observe an increase in

ventilatory amplitude; at P_{O_2} below 50 mmHg, ventilation appeared laboured, with a pronounced increase in ventilatory amplitude relative to that of rested fish.

Heart rate (H_f) was maintained independently of ambient P_{O_2} at oxygen tensions above 60 mmHg (Fig. 7.7). As P_{O_2} was reduced to 50 mmHg, a significant bradycardia became evident, with heart rate exhibiting a linear decline as P_{O_2} was further reduced. At 30 mmHg, H_f was reduced to 56% of that of rested snapper.

At oxygen tensions below 40 mmHg, cardiac arrhythmias were observed in 7 of the 8 fish, although the degree of arrhythmia was variable between individuals. In one individual, several instances of missed heart beats were also observed (Fig. 7.8).

During normoxia (140 mmHg), Int(H)/Int(V) varied between 0.8 and 1.4, with a modal value of 0.9. As P_{O_2} was reduced, the ratio increased in value, a function of both the bradycardia and the increase in ventilatory frequency, and became more broadly distributed. At 40 mmHg, the distribution midpoint and modal value was 2.1. Hence, while a minor degree of synchronisation may have occurred, with midpoints of the Int(H)/Int(V) distribution being closely associated with integers, the broad spread of values suggests cardiovascular and ventilatory rhythms were not tightly coupled during exposure to either normoxia or hypoxia (Fig. 7.9).

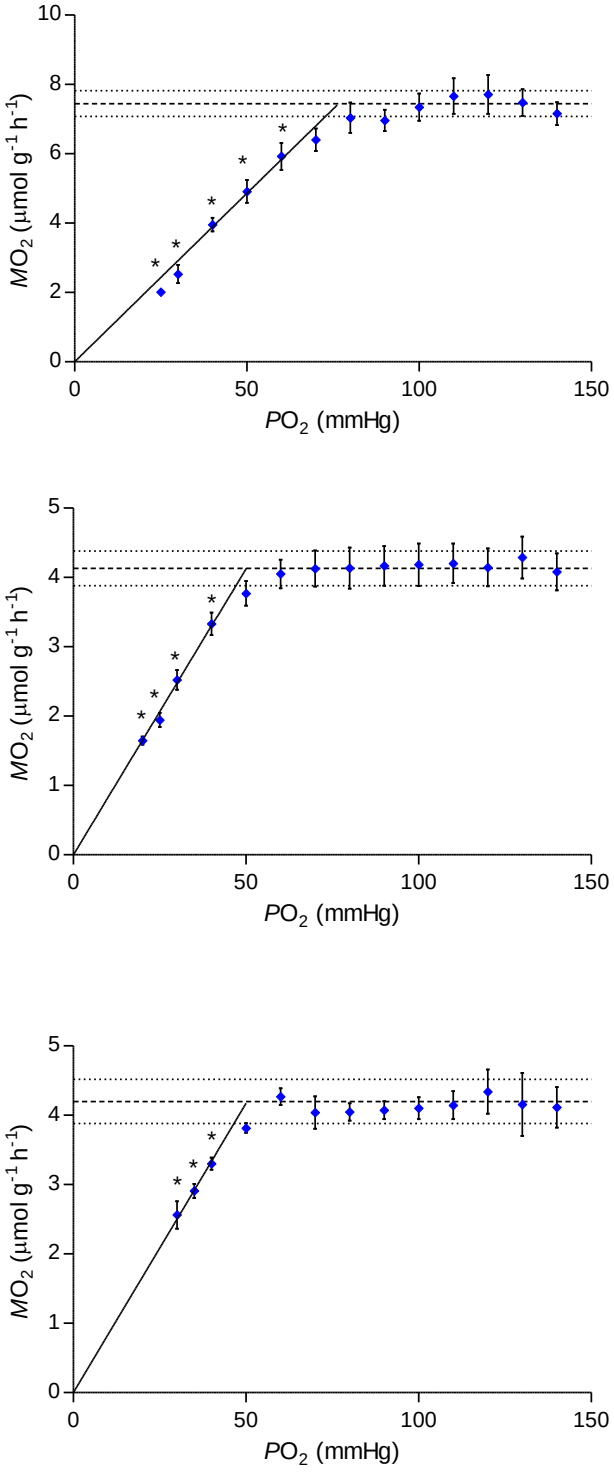


Figure 7.3 Routine oxygen consumption in a) 20 g, b) 150 g and c) 230 g snapper, exposed to progressively reduced oxygen tensions. Mean $MO_{2\ routine}$ and associated standard error determined for normoxic conditions are represented by the dashed and dotted horizontal lines, respectively. A linear regression (solid line) was fitted to those MO_2 values deemed significantly lower than $MO_{2\ routine}$ (denoted by *). P_{crit} was determined to be the intercept of this regression with $MO_{2\ routine}$. Regression equations: a) $MO_2 = 0.09718\ PO_2$, Sy.x = 0.8191; b) $MO_2 = 0.08244\ PO_2$, Sy.x = 0.3580; c) $MO_2 = 0.08343\ PO_2$, Sy.x = 0.3484.

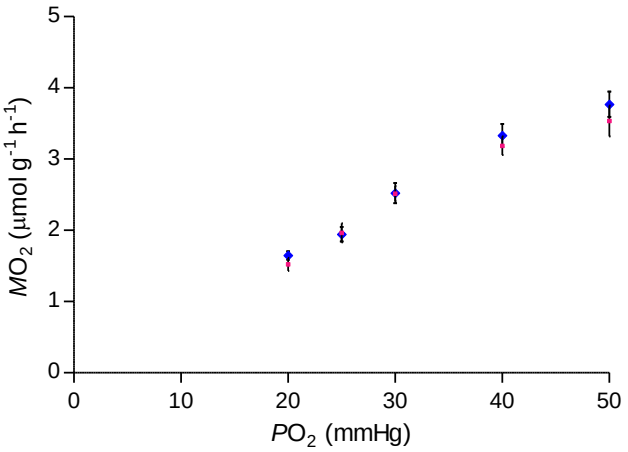


Figure 7.4. Routine oxygen consumption in 150-170 g snapper exposed to hypoxia induced by allowing fish to slowly consume available oxygen (◆) and by using nitrogen to rapidly reduce PO_2 to ~60 mmHg (■).

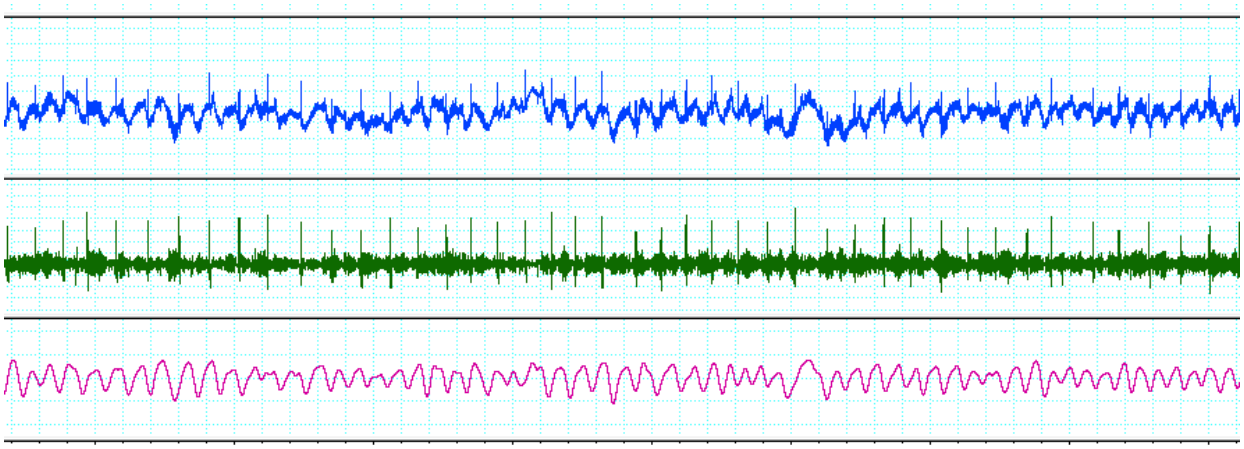


Figure 7.5. Representative ECG recording, depicting the raw ECG signal (top; blue), the ECG filtered for the clear display of heart beat (middle; green), and the ECG filtered for the clear display of ventilation (bottom; pink). Vertical lines denote 1 second time increments.

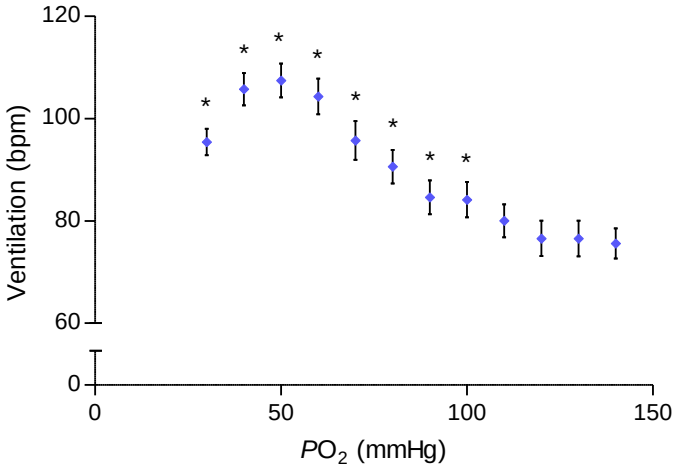


Figure 7.6. Ventilation rate in snapper during progressive reduction of ambient oxygen tension. * denotes a significant difference in rate to that of rested fish under normoxic (>140 mmHg) conditions.

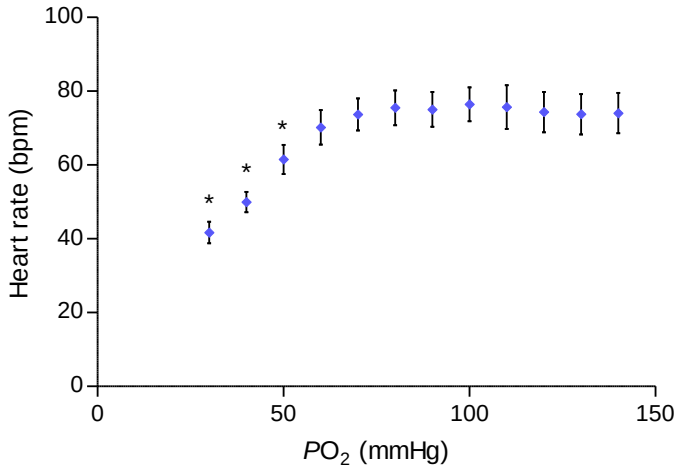


Figure 7.7. Heart rate in snapper during progressive reduction of ambient oxygen tension. * denotes a significant difference in rate to that of rested fish under normoxic (>140 mmHg) conditions.

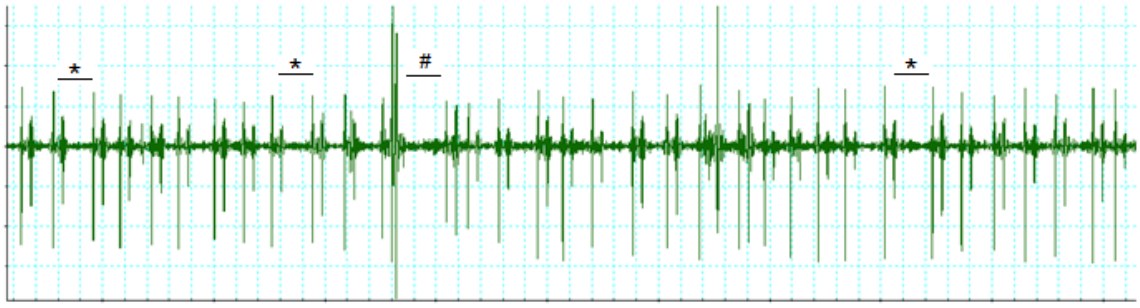


Figure 7.8. Example of a cardiac trace showing cardiac arrhythmia (*) and a missed beat (#) during significant, hypoxia-induced bradycardia.

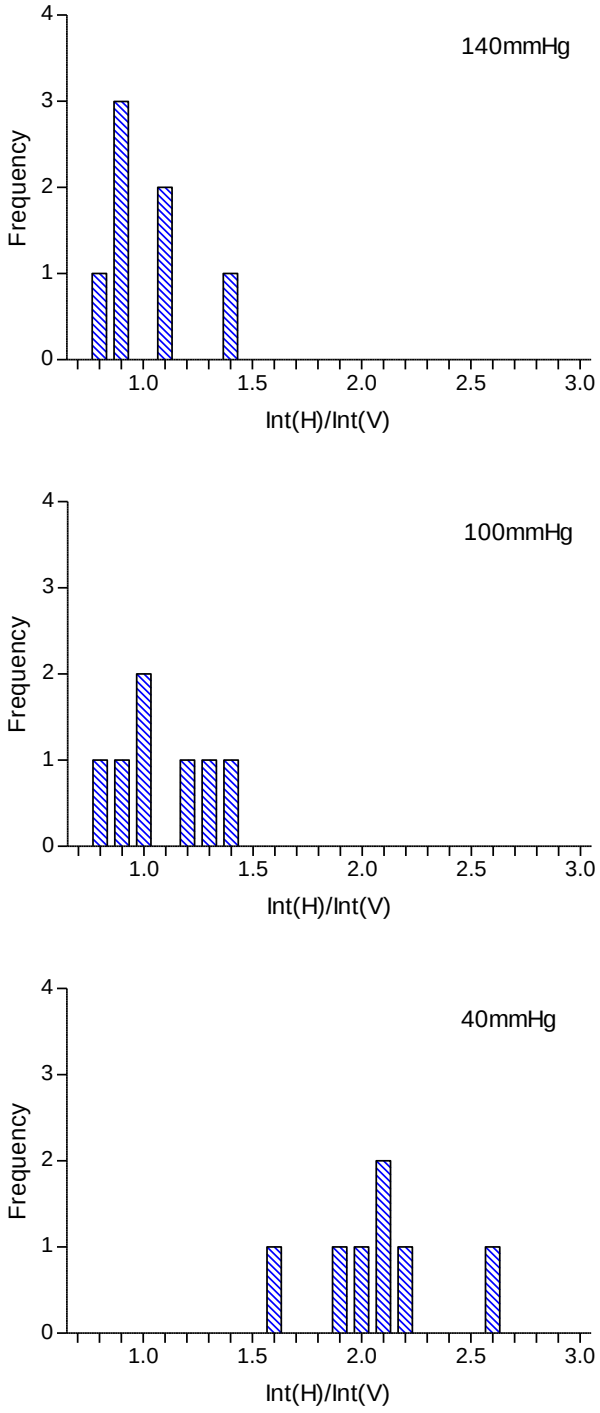


Figure 7.9. Frequency distributions for the Int(H)/Int(V) ratio in normoxic snapper, and during exposure to mild (100 mmHg) and severe (40 mmHg) hypoxia. Synchrony of cardiovascular and ventilatory rhythms would be implied by ratios distributions clustered about the integers.

7.3.3 The effect of hypoxia on swimming performance

Under normoxic conditions (150 mmHg), snapper exhibited a critical swimming speed of 5.10 ± 0.30 bl s^{-1}, consistent with that expected for fish of this size based on the allometric scaling of U_{crit} established in Chapter 3. Swimming performance was significantly impacted by reductions in PO_2 (Fig. 7.10; $p < 0.0001$); although performance tended to be reduced at all PO_2 levels below 150 mmHg, U_{crit} deteriorated rapidly at oxygen tensions below 80 mmHg. At 40mmHg, snapper were capable of 21% of the swimming performance attained under normoxic conditions.

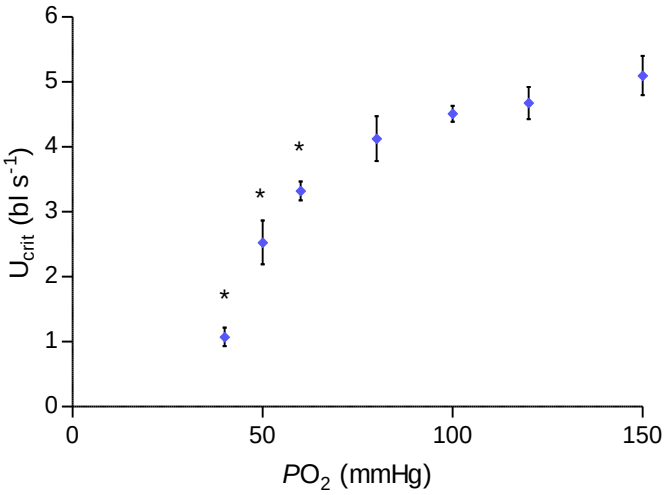


Figure 7.10. Critical swimming speed (U_{crit}) of juvenile snapper at different ambient oxygen partial pressures. * denotes a significant difference in U_{crit} that that attained by snapper at 150 mmHg

7.3.4 Effect of hypoxia on post-exhaustive oxygen consumption

There were no significant differences in $MO_{2\ routine}$ between the three recovery groups ($p = 0.3095$). Following exhaustive exercise, $MO_{2\ max}$ was significantly reduced in fish recovering at ambient oxygen tensions of 60-80mmHg (9.97 $\pm$ 0.50 µmol g^{-1} h^{-1}) compared with those exposed to 90-110 (12.76 $\pm$ 0.99 µmol g^{-1} h^{-1}) and 120-140mmHg (12.76 $\pm$ 0.44 µmol g^{-1} h^{-1}; $p=0.0119$).

Exposure to moderate hypoxia for an extended period following exhaustive exercise seemed to have a limited effect on the recovery of $MO_{2\ post\text{-}exercise}$. The shape of the $MO_{2\ post\text{-}exercise}$ recovery curves differed between the three PO_2 brackets; the normoxic group exhibited a more "classic" exponential decay of MO_2, however the two hypoxic treatments showed an abrupt reduction from MO_{2max} during the first 30 minutes, followed by a more linear decline in consumption rates. Despite the differences in the shape of the MO_2 recovery curves, there was no discernible trend in either the excess post-exercise oxygen consumption (EPOC; 7.9, 21.3 and 11.8 µmol g^{-1} for fish at 60-80, 90-110 and 120-140 mmHg, respectively) or the time required for $MO_{2\ post\text{-}exercise}$ to return to pre-exercise levels relative to recovery PO_2 (4.5, 7.0 and 3.5 hours for fish at 60-80, 90-110 and 120-140 mmHg, respectively; Fig. 7.11).

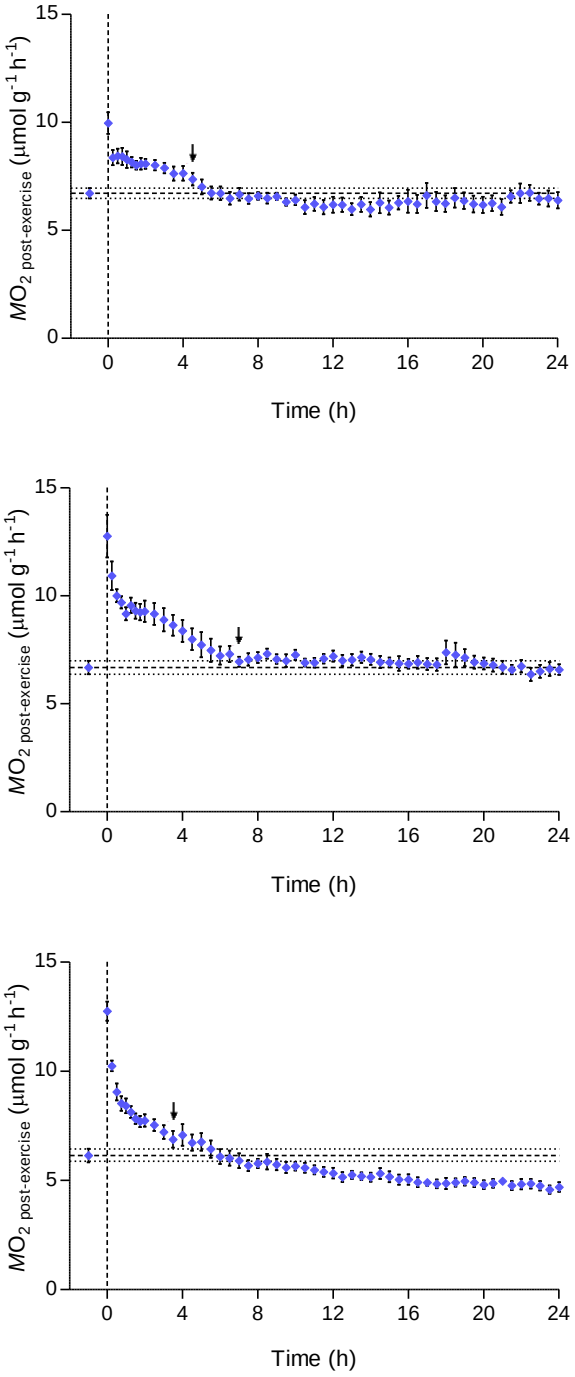


Figure 7.11. Post-exercise oxygen consumption in snapper maintained under three different levels of hypoxia: a) 60-80 mmHg, b) 90-110 mmHg and c)120-140 mmHg. The dashed vertical line represents the U_{crit} exercise test. MO_2 values at t = -1 hour represent $MO_{2\ routine}$ under normoxic conditions (120-140 mmHg), with the mean and standard errors transcribed across the figure for ease of comparison. For clarity, rather than indicate all of the values which are significantly different to $MO_{2\ routine}$, the point at which pre- and post-exercise metabolic rates are no longer significantly different are indicated with an arrow.

7.4 Discussion

7.4.1 Critical and terminal PO_2 in snapper

Snapper were able to maintain a constant $MO_{2\ routine}$ across a range of moderate to high ambient oxygen tensions, with compensation for the reduced availability of oxygen mediated by adjustments in cardiorespiratory function (see Section 7.4.2). At lower PO_2, snapper shifted to an oxygen-conforming state, in which MO_2 declined as a linear function of PO_2; the critical oxygen tensions defining this transition were 77 mmHg in 20 g snapper, and 50 mmHg in both 150 and 230 g snapper. While P_{crit} for the two larger size classes of fish are similar to critical tensions previously reported for snapper of similar size and acclimation temperature, the P_{crit} of the smaller fish is elevated compared with previous determinations (Cook et al., 2011; Patel, 2011; Cook and Herbert, 2012; Cook et al., 2013; Table 7.1). It is possible that these differences reflect the use of routine rather than standard metabolic rates for the determination of P_{crit} during the present study, since a number of species reduce their routine swimming activity during exposure to hypoxia as a means of reducing metabolic expenditure (Schurmann and Steffensen, 1994; Claireaux et al., 2000; Cech and Crocker, 2002; Herbert and Steffensen, 2005), which may give the appearance of a transition to an oxygen-conforming state at elevated PO_2. No attempt was made to qualify activity levels during the present study, for fear that the close observation of fish may incite confounding stresses that could have affected MO_2 (see Appendix Two). However, snapper have previously been observed to show a modest reduction (Cook and Herbert, 2012) or no change (Cook et al., 2011; Cook et al., 2013) in spontaneous swimming activity during the development of hypoxia, which would suggest that any overestimation of P_{crit} as a result of behavioural changes is likely to be small.

Alternatively, if the critical oxygen tensions for snapper detailed in Table 7.1 are plotted with respect to mass, a second possibility for the apparent elevation of P_{crit} in the smaller fish is suggested – that of size-specific variation in P_{crit} (Fig. 7.12). However, given the magnitude of the differences in the P_{crit} of ~20 g snapper obtained during the present study and by Patel (2011), it is difficult to conclude either in favour or opposition of any size effects. The possible influence of size on P_{crit} is explored further in the following section;

Table 7.1. Comparison of P_{crit} values derived during the present study with those previously reported for snapper, as well as for other fish species.

Species	Mass (g)	Temperature (°C)	Pcrit (mmHg)	Reference
Snapper	20	18	77	Present study
	150	18	50	Present study
	~200-250	18	39.8 ± 2.3	Cook et al., 2011
	225 ± 13	18	43.5 ± 4.5	Cook and Herbert, 2012
	10-30	19	53.7 ± 3.0	Patel, 2011
	70-150	15	43.1 ± 0.9	Patel, 2011
	150-300	21	52.0 ± 4.5	Cook and Herbert, 2013
Rainbow trout	115		90	Marvin and Heath, 1968
	250	14	50-53	McKenzie et al., 2007
	540-600	15-20	22-27	Ott et al., 1980
	150	10	21	Svendsen et al., 2012
Atlantic cod	150	5-15	25-47	Schurmann and Steffensen, 1997
	950-1800	2-10	23-30	Claireaux et al., 2000
Brook trout	50-250	10-15	80	Beamish, 1964*b*
Mulloway	340	22	38	Fitzgibbon et al., 2007
Carp	30-600	10-20	50	Beamish, 1964*b*
	1340-1650	10-25	20	Ott et al., 1980
Goldfish	17-290	10-20	45-50	Beamish, 1964*b*
	4	10-35	8-25	Fry and Hart, 1948
Bluegill	~118	25	120	Marvin and Heath, 1968
Plaice	10-15	10	45-60	Jobling, 1982

Species names: rainbow trout, *Oncorhynchus mykiss*; Atlantic cod, *Gadus morhua*; brook trout, *Salvelinus fontinalis*; mulloway, *Argyrosomus japonicas*; carp, *Cyprinus carpio*; goldfish, *Carassius auratus*; bluegill, *Lepomis macrochirus*; plaice, *Pleuronectes platessa.*

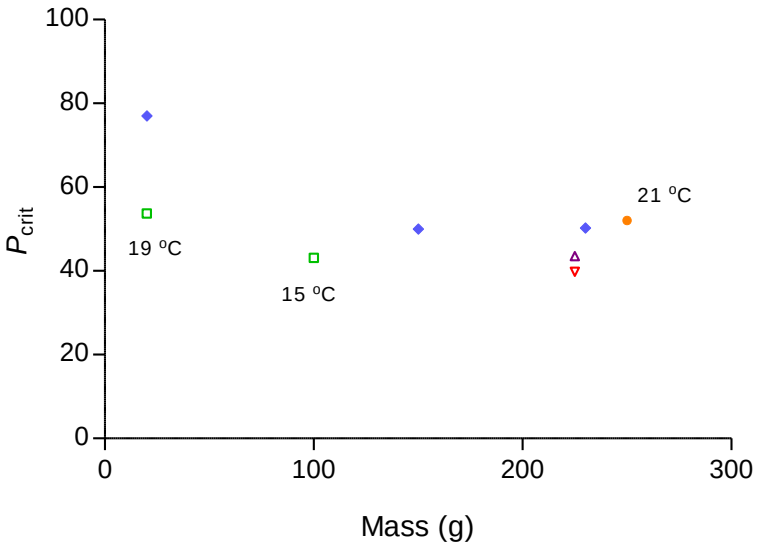


Figure 7.12. Summary of the critical oxygen tensions reported for snapper, in relation to body mass. Where experimental temperatures differ from 18 °C, these have been indicated beside the relevant data point. Data compiled from: ◆ present study; ▽ Cook et al. (2011); □ Patel (2011); △ Cook and Herbert (2012); ● Cook et al. (2013). Note that neither the error associated with P_{crit} nor fish size could be depicted due to difference in the format in which error was reported within the different studies (i.e. standard error, confidence intervals and total range were reported as measures of variation within various texts).

however, it is clear that our understanding of the relationship between mass and P_{crit} in snapper would benefit from the determination of critical oxygen tensions in fish spanning a greater size range, providing a greater context for the existing data.

P_{crit} is considered an indicator of hypoxia tolerance, since it represents the capacity of an animal to extract oxygen from the environment, relative to its metabolic demands (Richards, 2009). More athletic species, with their higher intrinsic oxygen requirements, have a tendency towards higher critical PO_2. However, comparative studies are few, and the inter- and intraspecific comparison of P_{crit} between studies is complicated by the multitude of factors which may influence P_{crit}, including temperature (Fry and Hart, 1948; Ott et al., 1980; Schurmann and Steffensen, 1997), size (Kalinin et al., 1993; Sloman et al., 2006; Everett and Crawford, 2010), nutritional and reproductive states (Pörtner and Grieshraber, 1993),

previous exposure to hypoxia (Everett and Crawford, 2010) and experimental differences (Ott et al., 1980; Ultsch et al., 1980). Bearing in mind these considerations, the P_{crit} reported for snapper, both previously and within the present study, suggest snapper to be moderately tolerant of hypoxia – more so than brook trout, but less so than Atlantic cod, carp or goldfish (Table 7.1).

The measure of critical oxygen tensions alone, however, may not provide a complete picture of the tolerance of a species to hypoxia, since many species are able to depress (to varying degrees) their standard metabolic rates, via the controlled suppression of protein synthesis, transmembrane ion transport, RNA transcription, gluconeogenesis and other anabolic processes (Boutilier, 2001; Richards, 2009). Suppression of metabolic demand at the cellular level reduces the reliance on anaerobic metabolism, extending survival time when ambient oxygen tensions decline below P_{crit} (Richards, 2009). For example, in the Oscar cichlid (*Astronotus ocellatus*), $MO_{2\ standard}$ is reduced at PO_2 below 31 mmHg, yet an increase in plasma lactate – indicative of the recruitment of supplementary anaerobic metabolism – was evident only at PO_2 below 10 mmHg (Muusze et al., 1998). Hypoxia tolerant species such as the goldfish and tilapia (*Oreochromis mossambicus*) may survive prolonged exposure to severe hypoxia (>1 hour at 0-7 mmHg) through the depression of their respective metabolic rates by 70 and 55% (van Waversveld et al., 1989; van Ginneken et al., 1997). Crucian carp (*Carassius carassius*) may similarly depress their metabolic rate, which in combination with their ability to mitigate the adverse effects of lactacidosis through the production of ethanol, enables survival under anoxic conditions for a number of weeks (Vornanen et al., 2009). Whilst the ability of snapper to reduce their standard metabolic rate at oxygen tensions below P_{crit} is unknown, the accumulation of lactate within the plasma and cardiac and hepatic tissues at oxygen tensions of 22-24 mmHg (Cook et al., 2011; Cook and Herbert, 2012) indicates that any metabolic depression that snapper may be capable of is insufficient to reduce metabolic expenditure to levels which may be supported aerobically at such low oxygen tensions.

Terminal PO_2, defined as the PO_2 at which snapper exhibited urgent, escape-type behaviour which preceded a loss of equilibrium are reported to provide a more complete picture of the hypoxia tolerance of snapper. Terminal PO_2 is a relatively arbitrary measure of hypoxia tolerance, considering the subjective nature of the experimental endpoint (i.e. what

constitutes an escape response?), the potential for dissociation between the expression of behaviour and physiological status (Farrell and Richards, 2009), and further, that in the absence of metabolic depression, survival at oxygen tensions below P_{crit} is a function of time (Farrell and Richards, 2009; Richards, 2009). Nonetheless, P_{term} is of value in describing the ambient oxygen tensions that posed an imminent threat to the survival of snapper. The PO_2 at which escape behaviours were observed (29 and 22 mmHg in 20 and 150 g snapper) are similar to those reported by Cook et al. (2011) and Cook and Herbert (2012) as inciting avoidance behaviour in snapper (approximately 22-24 mmHg). Together, the loss of equilibrium observed during the present study and the accumulation of lactate observed post-avoidance (Cook et al., 2011; Cook and Herbert, 2012) indicate that ambient oxygen tensions of 20-30 mmHg will be lethal to snapper that are unable to escape to areas of higher oxygen tension. Although neither Cook et al. (2011) nor Cook and Herbert (2012) describe any urgency to the avoidance behaviours, Herbert et al. (2011) demonstrated that for Atlantic cod, voluntary entry and residence time within hypoxic water was dependent on the presence of an oxygen refuge; hence, the oxygen refuge provided by the alternate choice chamber in the studies of Cook and colleagues may have reduced the stress experienced by snapper, despite the extent of oxygen depletion. In contrast, the inability of snapper to relocate from oxygen-depleted waters during the present study may have led to the escalation of their escape behaviours, with burst swimming likely a "last-resort" attempt to escape the hypoxic environment and impending asphyxia.

7.4.1.1 The effects of size on hypoxia tolerance

Smaller snapper exhibited a higher P_{crit} than did larger fish, with critical oxygen tensions of 77 mmHg in 20 g fish, and 50 mmHg in 150 and 230 g fish. Whilst it is possible that these differences in P_{crit} result from variation in the behavioural response to hypoxia, since changes in spontaneous activity may imply an artificially high P_{crit}, it is also possible that they represent inherent size-specific variation in hypoxia tolerance. That smaller snapper exhibited higher terminal PO_2 values than did larger fish would further suggest that smaller snapper are more sensitive to hypoxia. Higher critical oxygen tensions have previously been suggested for smaller snapper (Patel, 2011), however confounding temperature variation precluded more definite conclusions as to the association between size and hypoxia tolerance.

Since the allometric scaling of metabolism has been well documented in fish (see Chapter 3), it might be expected that hypoxia tolerance would demonstrate a degree of size-dependence (Everett and Crawford, 2010). Indeed, a number of studies have reported size-specific differences in hypoxia tolerance, typically characterised by smaller individuals being less tolerant than larger conspecifics. For example, larger Oscar cichlids are more hypoxia tolerant than are smaller fish, both in terms of P_{crit} (Sloman et al., 2006) and survival time (Almeida-Val et al., 2000). Similarly, P_{crit} has been negatively correlated with mass in gulf killifish (*Fundulus grandis*; Everett and Crawford, 2010) and wolffish (*Hoplias malabaricus*; Kalinin et al., 1993), as have lethal oxygen tensions in red seabream (*Pagrus major*; Ishibashi et al., 2005). Both P_{crit} and the oxygen tensions at which damelsefishes (Pomacentridae) lose equilibrium were significantly correlated with mass, with P_{crit} reduced from 45 to 35 mmHg as mass increased from 10 mg to 40 g (Nilsson and Östlund-Nilsson, 2008). In contrast, tolerance has been found to be independent of size in Atlantic cod (Plante et al., 1998) and nile tilapia (*Oreochromis niloticus*; Verheyen et al., 1994). Further, while the critical PO_2 of sharpsnout seabream (*Diplodus puntazzo*) is independent of size, survival time favours smaller fish (Cerezo and Garcia, 2004).

The mechanistic basis for the scaling of hypoxia tolerance remains elusive, with scaling relationships used to argue for a greater tolerance in both small and large fish (reviewed by Nilsson and Östlund-Nilsson, 2008). The constraints imposed by hypoxia on metabolism are generally considered to be amplified in smaller fish as a function of their higher mass-specific metabolic rate (Almeida-Val et al., 2000; Sloman et al., 2006). Conversely, it has been suggested that since gill surface area demonstrates negative allometry, the smaller relative surface area in large fish should confer a greater sensitivity to hypoxia (Robb and Abrahams, 2003). Alternatively, Nilsson and Östlund-Nilsson (2008) suggest that since the scaling exponents of $MO_{2\ standard}$ and gill surface area are similar, and gill diffusion distance is independent of size, the capacity for oxygen uptake is closely matched to metabolic demand, and hence P_{crit} should be independent of size. However, oxygen uptake is a function not only of respiratory surface area, but also of ventilatory water volume, gill perfusion and blood oxygen-carrying capacity (Perry et al., 2009). While possible differences in the ventilatory response of fish of different size have not been investigated, it is of note that differences in the species-specific response have not been correlated with hypoxia tolerance (Perry et al., 2009). Similarly, the role of the cardiovascular system in any scaling of tolerance is unknown

(Nilsson and Östlund-Nilsson, 2008). Blood haemoglobin content, haemoglobin affinity and erythrocyte recruitment are also unlikely to be involved, since they either scale isometrically with size, or do not correlate with hypoxia tolerance (Nilsson and Östlund-Nilsson, 2008).

Since the only avenue of ATP production under anaerobic conditions is glycolysis, survival time at oxygen tensions below P_{crit} depend on the magnitude of anaerobic fuel reserves, the tolerance of the tissues for metabolic end-products and the rate at which anaerobic reactions progress – itself a determinant of metabolic rate and the degree by which anaerobic glycolysis must supplement the aerobic generation of ATP (Nilsson and Östlund-Nilsson, 2008; Richards, 2009; Svendsen et al., 2012). To this end, the higher metabolic rate of smaller fish will require glycogen be consumed and end-products accumulate at greater rates than in larger fish, severely limiting the survival time of smaller fish (Nilsson and Östlund-Nilsson, 2008).

7.4.2 The cardiorespiratory response to hypoxia

Changes in ventilatory frequency are arguably the most important of the physiological responses to reduced oxygen availability (Perry et al., 2009). Although most fish species are capable of detecting and actively avoiding hypoxic environments, many tend to show avoidance only at more severe levels of oxygen depletion (Diaz and Breitburg, 2009; Cook et al., 2011). Rather, the primary response of most fish species to mild hypoxia is an increase in ventilatory minute volume (V_m) which serves to increase the net volume of oxygen presented at the respiratory surface, maximising the transbranchial PO_2 gradient in an attempt to preserve arterial oxygen content and hence aerobic metabolic capacity (Holeton and Randall, 1967; Randall, 1982; Perry et al., 2009). This increase in minute volume is mediated through changes in ventilatory frequency and/or ventilatory stroke volume, with both the relative contribution of each to the overall change in minute volume and the PO_2 threshold at which changes are initiated being species-specific (Holeton and Randall, 1967; Marvin and Heath, 1968; Hughes and Saunders, 1970; Hughes, 1973; Borch et al., 1993; Perry et al., 2009). The compendium by Perry et al. (2009) demonstrates that for most teleost species, an increase in ventilatory stroke volume is the dominant means of increasing minute volume, coupled with more modest changes in frequency. For example, hypoxic rainbow trout may increase

ventilatory stroke volume 470-760%, with a concurrent increase in ventilatory frequency of only 50-170% (Holeton and Randall, 1967; Hughes and Saunders, 1970).

Similarly, snapper responded to hypoxia with an increase in both ventilatory frequency and stroke volume, with the latter appearing to dominate the response. Although the methods used in the present study did not permit the quantification of any change in stroke volume, it was possible to visually observe an increase in ventilatory amplitude – a common proxy for stroke volume (Perry et al., 2009). Under normoxic conditions, the ventilatory amplitude was small, with opercular movements subtle and often difficult to observe; however, as $P\mathrm{O}_2$ was reduced to 40 mmHg, ventilation appeared laboured, with amplitude estimated to have increased several-fold, and ventilation frequency increased by 40%.

The metabolic costs associated with ventilation are thought to comprise up to 20% of $M\mathrm{O}_{2\ \mathrm{standard}}$ (Marvin and Heath, 1968; Heath, 1973), hence the significant increase in ventilatory effort observed in response to hypoxia is presumably associated with a sizeable metabolic cost. Interestingly, while some species demonstrate an increase in $M\mathrm{O}_2$ during exposure to mild to moderate hypoxia (Beamish, 1964*b*; Marvin and Heath, 1968; Ott et al., 1980; Rantin et al., 1992; Verheyen et al., 1994), others do not (Ott et al., 1980; Verheyen et al., 1994); notably, Cook and Herbert (2012) and Cook et al. (2013) observed no significant increase in $M\mathrm{O}_{2\ \mathrm{standard}}$ in hypoxic snapper. It is possible that the concurrent reduction in other metabolic costs masks any increase in costs incurred by the ventilatory musculature, or that the costs are relatively small in the context of total metabolic expenditure and thus any increase difficult to identify. Ultimately, $P\mathrm{O}_2$ becomes sufficiently low that the metabolic costs associated with the required increase in ventilatory effort exceed the benefit derived from the increase in water flow, and ventilatory failure occurs (Perry et al., 2009). This decline in ventilatory function is often seen to occur at $P\mathrm{O}_2$ similar to the critical $P\mathrm{O}_2$ (Holeton and Randall, 1967; Rantin, 1992; Perry et al., 2009); indeed, peak ventilation rate was coincident with P_{crit} at 50 mmHg.

In addition to hyperventilation, changes in cardiovascular function are critical to ensuring efficient oxygen uptake and distribution to the tissues during periods of environmental oxygen limitations (Farrell, 2007*a*; Gamperl and Driedzic, 2009). An increase in systemic blood pressure and an adrenergically-mediated branchial vasodilation promote the recruitment of unperfused gill lamellae, increasing the effective respiratory surface area and hence the capacity for oxygen uptake (Booth, 1979; Randall, 1982; Farrell, 2007*b*). Systemic

vasoconstriction functions as an oxygen-sparing strategy, preserving blood oxygen content for essential organ function (Gamperl and Driedzic, 2009; Janssen et al., 2010), in particular cardiac function, since the terminal position of the heart in the systemic circulation renders it dependent on venous oxygen content (Farrell, 2007*b*; Gamperl and Driedzic, 2009). The recruitment of additional erythrocytes from the spleen increases the oxygen carrying-capacity of the blood (Yamamoto et al., 1983; Cook and Herbert, 2012).

The most prominent feature of the cardiovascular response to hypoxia, however, is a pronounced bradycardia, mediated by an increase in vagal tone, and accompanied by an increase in cardiac stroke volume of up to 3-fold, resulting in part from the increased end-diastolic volume (via the Frank Starling mechanism), and which compensates for the effects of bradycardia on cardiac output (Q) (Holeton and Randall, 1967; Randall, 1982; Farrell, 2007*b*; Gamperl and Driedzic, 2009). The bradycardia typically develops at lower oxygen tensions than those that incite changes in ventilatory function (Holeton and Randall, 1967; Marvin and Heath, 1968; de Salvo Souza et al., 2001; Gamperl and Driedzic, 2009), although both the threshold $P\mathrm{O}_2$ at which bradycardia is initiated and the magnitude of the response show significant species-specific variation (Gamperl and Driedzic, 2009). This variation is thought to be correlated with the ecology and general hypoxia tolerance of a species; for example rainbow trout, dourado (*Salminus maxillosus*) and smallmouth bass (*Micropterus dolomieu*) – active species that inhabit well-oxygenated environments – develop bradycardia at 110 mmHg (Marvin and Heath, 1968), 90 mmHg (Furimsky et al., 2003) and 70 mmHg (de Salvo Souza et al., 2001), respectively, while hypoxia tolerant species such as the tench (*Tinca tinca*) and largemouth bass (*Micropterus salmoides*) exhibit a decline in heart rate at oxygen tensions <40 mmHg (Marvin and Heath, 1968; Furimsky et al., 2003). That snapper exhibit a bradycardia at oxygen tensions below 60 mmHg is therefore consistent with the emerging picture that snapper are moderately tolerant of hypoxia. It is of note however, that Janssen et al. (2010) reported a resting heart rate for anaesthetised snapper of 39 bpm, with bradycardia developing only as oxygen tensions reached 31 mmHg; although the reasons for the differences between the two studies are unknown, it is probable that they reflect the use of anaesthetised and routinely active fish in the respective studies.

The purpose of the hypoxic bradycardia remains somewhat enigmatic, however it appears that rather than facilitate oxygen uptake, it is concerned with the maintenance of cardiac function, since hypoxia has been shown to have a direct effect on the contractile

function of myocytes (Steffensen and Farrell, 1998). Farrell (2007*b*) discussed several potential mechanisms by which bradycardia may preserve both oxygen delivery to and utilisation by the heart during hypoxia, including an increase in the contractile function of the myocytes, since force development is an inverse function of contraction velocity; an increase in the potential for oxygen to diffuse to the myocardium, since maximum oxygen extraction occurs during diastole, and the increase end-diastolic volume stretches the trabeculae, reducing diffusion distances; and reduced myocardial oxygen demand, due to a decrease in the rate of ventricular pressure development.

The development of cardiac arrhythmia during exposure to hypoxia has been demonstrated in several fish species (Steffensen and Farrell, 1998; Iversen et al., 2010), including snapper (Janssen et al., 2010). Steffensen and Farrell (1998) observed the development of arrhythmia in coronary-ligated (but not control) trout during exposure to severe hypoxia, suggesting arrhythmia is a direct consequence of oxygen limitation at the myocyte. In isolated tissues, hypoxia has been shown to incite arrhythmia through the impairment of ion channel function, which results in partial membrane depolarisation and altered cardiac action potential development (Shimoda and Polak, 2011).

Several studies have documented a degree of synchrony between the cardiac and ventilatory cycles, often referred to as cardiorespiratory coupling (CRC), during the development of hypoxia (Randall and Smith, 1967; Berchick et al., 1987; Borch et al., 1993). Since both blood flow through the afferent branchial vessels and water flow across the gill lamellae are highly pulsatile, synchrony of the cardiac and ventilatory cycles would appear to increase the efficiency of oxygen uptake, by ensuring that peak blood flow through the gill is coincident with the maximum presentation of oxygen at the respiratory surface (Taylor et al., 1999). The possibility of cardiorespiratory synchrony was investigated by calculating the frequency distribution of the ratios of the heart beat-to-beat and ventilatory beat-to-beat intervals, Int(H)/Int(V). Although the vague grouping of the distribution about integer ratio values suggests a degree of synchrony was evident, the lack of any tight association, in particular in hypoxic fish, suggests snapper to not exhibit significant CRC during exposure to hypoxia.

7.4.3 The effect of hypoxia on swimming performance

That U_{crit} is reduced in snapper exposed to hypoxic conditions is consistent with the reduced swimming performance previously observed for other fish species (Jones, 1971; Kutty and Saunders, 1973; Bushnell et al., 1984; Dutil et al. 2007; Fitzgibbon et al. 2007; Jourdan-Pineau et al., 2010, Petersen and Gamperl, 2010; Zhang et al., 2010; Fu et al., 2011; McKenzie et al., 2012). Considerable variation exists, however, as to the oxygen tensions at which swimming performance becomes impaired, and the magnitude of subsequent impairment, with the sensitivity of performance apparently correlated with the ecology of the animal and its tendency to encounter hypoxia within its environment. For example, Jones (1971) documented a 34-42% decline in the U_{crit} of rainbow trout exposed to ambient oxygen tensions of ~78 mmHg, although Bushnell et al. (1984) reported only a 25% decrease at 40 mmHg. Similarly, U_{crit} in Atlantic cod was reduced by a third when exercised at an ambient PO_2 of 63-70 mmHg (Petersen and Gamperl, 2010). In contrast, fish that are more frequently exposed to a degree of hypoxia are capable of maintaining performance at greatly reduced oxygen tensions. The critical swimming performance of catfish (*Silurus meridionalis*) declined 27% when ambient PO_2 was reduced to 37 mmHg, falling to 47% of normoxic U_{crit} at 12 mmHg (Zhang et al., 2010), while U_{crit} in carp was reduced 40% during locomotion at 30 mmHg. Fu et al. (2011) found goldfish to be capable of 62-67% of their normoxic U_{crit} performance at oxygen tensions of only 14 mmHg. During the present study, U_{crit} was reduced 19% as PO_2 was reduced to 80 mmHg, falling to 50% of the normoxic swimming performance at 50 mmHg. The critical swimming performance of snapper therefore appears to be moderately sensitive to hypoxia.

The effects of hypoxia on swimming performance are readily explained by the constraints imposed on metabolic scope. Since cardiovascular and ventilatory performance is maximal during swimming at or near U_{crit}, the fish has no capacity to augment oxygen uptake in the face of a reduced branchial diffusion gradient, and hence $MO_{2\ max}$ and aerobic scope decline with PO_2, limiting the provision of oxygen to metabolically active tissues (Fitzgibbon et al., 2007). In particular, it is likely that cardiac performance plays a key role in limiting sustained or prolonged swimming performance during exposure to hypoxic conditions, due to its dependence on venous oxygen content; an increase in oxygen extraction by the working myotomal musculature would therefore compromise myocardial oxygen supply, which is

already tenuous under hypoxic conditions (Farrell, 2007*a*, 2007*b;* Steinhausen et al., 2008). This will be especially true for snapper, which lack a coronary blood supply to the myocardium (Cook and Herbert, 2012).

Although $\dot{M}O_{2\ max}$ was not measured during the present study, Cook et al. (2011) and Patel (2011) have previously demonstrated that for snapper, $\dot{M}O_{2\ max}$ shows signs of impairment at ambient oxygen tensions of approximately 105-110 mmHg, with aerobic metabolic scope reduced 10-24%. During the present study, U_{crit} was slightly, though not significantly, reduced at 100 and 120 mmHg (12 and 8%, respectively). Although the reduction in metabolic scope reported by Cook et al. (2011) and Patel (2011) exceeded that observed for U_{crit} during exposure to moderate hypoxia (~60-100 mmHg), metabolic scope and U_{crit} were similarly reduced by approximately 82 and 79% of their respective normoxic performance, at ambient tensions of 45 and 40 mmHg. Interestingly, snapper were capable of limited swimming activity at 40 mmHg (U_{crit} 1.07 bl s^{-1}), an apparent contradiction of the critical oxygen tensions determined for snapper during the present study, since the scope for activity framework dictates that at ambient oxygen tensions below P_{crit}, the scope for aerobic metabolism to support such activity is nil (Fry, 1947). Cook and Herbert (2012) and Cook et al. (2013) also observed routine activity of 0.5-0.6 bl s^{-1} in snapper exposed to PO_2 below P_{crit}. While these observations might suggest that existing estimates of P_{crit} are overestimated, it is also possible that such activity could be fuelled by the anaerobic production of ATP. Although the voluntary consumption of anaerobic fuel reserves during hypoxia may seem counterintuitive, there may be an adaptive value in such behaviour, through increasing the chances of successfully relocating to an area of higher ambient PO_2.

7.4.4 The effects of hypoxia on recovery from exhaustive exercise

During exhaustive exercise, the recruitment of the glycolytic WM results in the significant disturbance of metabolic, acid-base, ionic, osmotic, and hormonal balance (Wood, 1991; Milligan, 1996). The elevation of $\dot{M}O_{2\ post\text{-}exercise}$, described as EPOC, represents the aerobic metabolic cost of correcting these perturbations, including the resynthesis of PCr, ATP and glycogen, recharging intracellular oxygen reserves and the restoration of homeostasis (Scarabello et al., 1991, 1992; Wood, 1991). Although the effects of hypoxia on

swimming performance have been well-documented, the possible effects of hypoxia on EPOC and recovery from exercise have not previously been studied (Domenici et al., 2013).

Exposure to mild to moderate hypoxia during recovery from exhaustive exercise appeared to have relatively little impact on the recovery of $\dot{M}O_{2\ \text{post-exercise}}$. $\dot{M}O_{2\ \text{max}}$ was reduced 22% in snapper exposed to 60-80 mmHg during recovery, similar to the 25-30% and ~35% reduction in $\dot{M}O_{2\ \text{max}}$ reported by Patel (2011) and Cook et al., (2011) respectively, in snapper exposed to ambient PO_2 of 75 mmHg. The consequences of this reduction in the capacity for oxygen uptake remains somewhat unclear; in part because the establishment of post-exercise $\dot{M}O_{2\ \text{routine}}$ below those determined pre-exercise (similar to that observed in Chapter 3) meant no discernible trends in EPOC or recovery time with respect to ambient PO_2 were evident. However, if EPOC is estimated using post-exercise rather than pre-exercise $\dot{M}O_{2\ \text{routine}}$ (i.e. 6.0, 6.8 and 5.5 μmol g^{-1} h^{-1} at 60-80, 90-110 and 120-140 mmHg, respectively), then EPOC is similar across the three groups (at 19.0, 18.7 and 17.6 μmol g^{-1} for 60-80, 90-110 and 120-140 mmHg), although there remains substantial variation in recovery time (13.0, 20.5 and 16.1 hours for 60-80, 90-110 and 120-140 mmHg). The similarity in the EPOC suggests a similar magnitude of disturbance induced by exercise, and hence in the cost of restoring homeostasis, consistent with the same U_{crit} protocol and conditions being used across the three groups. It might have been expected that EPOC would be slightly elevated in hypoxic fish, reflective of the metabolic costs associated with an increased ventilatory effort at low PO_2; however, while there is a slight tendency towards higher EPOC in hypoxic fish, the differences are minimal, consistent with absence of an obvious increase in $\dot{M}O_2$ during the determination of P_{crit}. As mentioned, however, it is important to note that the possible concurrent reduction of other costs (i.e. spontaneous activity) may mask any increased metabolic costs associated with ventilatory effort.

In a similar study, Svendsen et al. (2012) investigated the effects of moderate hypoxia on the recovery of rainbow trout from hypoxia-induced anaerobiosis. Presumably, recovery from anaerobiosis occurs via similar mechanisms irrespective of the inciting stressor, hence parallels between the effects of hypoxia on EPOC and EPHOC (excess post-hypoxia oxygen consumption) may be drawn. In trout exposed to hypoxia (50 mmHg) during recovery, the peak post-hypoxia $\dot{M}O_2$, $\dot{M}O_{2\ \text{peak,}}$ was reduced 50% compared with $\dot{M}O_{2\ \text{peak}}$ of trout recovering under normoxic conditions. Total EPHOC was unchanged, however, in contrast to the present study, the period of time required for $\dot{M}O_2$ to return to pre-hypoxia rates was

prolonged in hypoxic fish; presumably, the constraints on oxygen uptake limited that rate at which corrective processes could occur, prolonging recovery.

Although the possible effects of hypoxia on the duration of post-exercise recovery remain inconclusive, the lack of an obvious extension to recovery time in snapper recovering at 60-80 mmHg suggests that the PO_2 increments used were not severe enough to significantly impair metabolic recovery. The recovery conditions used by Svendsen et al (2012) were more severe than those of the current study, and resulted in a 70% reduction in metabolic scope, compared with a 50% reduction observed in snapper. Also of note in the present study is the difference in the shape of the $MO_{2\ recovery}$ curves, with normoxic fish exhibiting a classic exponential decay and hypoxic fish showing a sudden decline in MO_2 almost immediately after introduction to the hypoxic environment, which was followed by a linear decline towards $MO_{2\ routine}$. Although the significance of differently-shaped $MO_{2\ recovery}$ curves is unknown, they appear to permit the same total EPOC over a similar timeframe, despite the initial limitation of $MO_{2\ max}$.

Interestingly, the 50% reduction in metabolic scope at 60-80 mmHg suggests P_{crit} is lower for smaller fish than that implied in Section 7.3.1, since there would be little to no scope to increase MO_2 post-exercise were P_{crit} in the range of 77 mmHg.

7.5 Conclusions

Snapper exhibit a moderate tolerance to hypoxia, demonstrating a classic teleost response to low oxygen availability. Snapper are oxygen-regulators, maintaining $MO_{2\ routine}$ across a range of ambient oxygen tensions, before becoming dependent on ambient PO_2below a critical oxygen tension. Pcrit was determined to be 77 mmHg in 20 g fish, and 50 mmHg in both 150 and 230 g fish; hypoxia tolerance in snapper therefore appears to be size dependent, favouring larger individuals. Exposure to severe hypoxia (20-30 mmHg) was associated with escape behaviours, characterised by burst activity and obvious distress, and was soon followed by a loss of equilibrium. Together with reports of the accumulation of lactate within the tissues at these PO_2 (Cook and Herbert, 2012), these observations indicate oxygen tensions of 20-30 mmHg will prove lethal to snapper unable to relocate to areas of higher PO_2.

The capacity for oxygen-regulation was mediated by changes in both cardiovascular and ventilatory function. In particular, both ventilatory frequency and stroke volume increased at $P\mathrm{O}_2$ below 100 mmHg, with the latter appearing to dominate the ventilatory response. A reflex bradycardia developed at oxygen tensions below 60 mmHg, with heart rate reduced by half at 30 mmHg, when cardiac arrhythmia also became evident, possibly an indication of impending cardiac failure. There was no evidence of cardiorespiratory coupling as a means of increasing the efficiency of oxygen uptake during periods of oxygen limitation.

The constraints imposed by hypoxia on metabolic scope directly impact the capacity for exercise, with critical swimming performance reduced at $P\mathrm{O}_2$ below 80 mmHg, with snapper capable of just 21% of their normoxic swimming performance at 40 mmHg. Exposure to hypoxia (60 to 80 mmHg) during recovery from exhaustive exercise constrained $M\mathrm{O}_{2\,\mathrm{max}}$, and while it did not appear that this affected either EPOC or the period required for $M\mathrm{O}_2$ to return to routine levels, a high degree of behavioural variation post-exercise precludes any firm conclusions as to the effects of hypoxia on the duration of the recovery period.

CHAPTER 8
GENERAL DISCUSSION

8.1 Thesis objectives: understanding the exercise physiology of snapper

Internationally, the increasing demand for fish and fisheries products, in combination with socioeconomic pressure for industry expansion, is placing considerable pressure on wild fish stocks, more than 80% of which are currently considered to be maximally- or over-exploited (Botsford et al., 2001; Zabel et al., 2003; FAO, 2009; Worm et al., 2009). Since the capacity to stimulate growth through increased landings is therefore severely constrained, adding value to the existing catch by way of improvements in product quality may offer an alternative means of improving financial return, and of increasing yield through reducing waste associated with limited processing options and/or shelf-life (Huss, 1995; Forgan, 2009). There is a rapidly growing body of literature demonstrating the importance of the peri-mortem physiology of an animal for the post-mortem condition of its tissues, which is in turn a key determinant in product quality (Wells, 1987; Lowe et al., 1993; Huss, 1995; Berg et al., 1997; Erikson et al., 1997; Sigholt et al. 1997; Jerrett et al., 1998; Thomas et al., 1999; Robb et al., 2000; Skjervold et al., 2001; Black et al., 2004; Roth et al., 2006; Bosworth et al., 2007; Oehlenschlager and Rehbein, 2009; Tuckey et al., 2010). The potential benefits of this knowledge are evident within the aquaculture industry, where the development of rested harvesting techniques has led to significant improvements in product quality. As most commercial fishing methods result in the stress, fatigue and/or injury of the catch, targeted amendments to the capture process (for example, through gear design and/or harvesting practices) aimed at mitigating the stress experienced may therefore yield similar improvements in product quality (Lowe et al., 1993). Further, as the nature and severity of stressors experienced during capture are crucial in determining the survival of juvenile and non-target species escaping from the net or discarded as bycatch, such amendments may also

confer improvements in sustainability (Davis, 2002; Suuronen, 2005; Broadhurst et al., 2006).

An understanding of the physiology of the target fish species is required for the effective implementation of alterations in gear design or harvest protocols. For example, in trawl-based fisheries, the exercise physiology of a species – in particular, its swimming capacity – is key in determining its interaction with fishing gears, with implications for overall yield, the efficacy of by-catch reduction devices (BRDs), and of the condition of fish at landing (Wardle, 1993; He, 1993; Breen et al., 2004; Winger et al., 2010). The objective of the present study was therefore to explore the exercise physiology of the snapper, *Pagrus auratus*. Snapper is already a well-regarded, export-quality product, which is suitable for use as high-end products such as sashimi when in top condition. However, considering the prevalence of trawling as a harvest method within the snapper fishery (i.e. trawling accounts for 40% of the total commercial catch in SNA1; Ministry for Primary Industries, 2013*a*), and concerns regarding the status of some stocks (Ministry for Primary Industries, 2013*a*, 2013*c*), it would seem that there is potential for improvements in both the quality and sustainability of New Zealand's snapper fishery. The preceding chapters describe several discrete studies pertaining to the exercise physiology of snapper, including characterising swimming capacity, the physiological consequences of exercise and capture, and the potential for metabolic recovery. The present discussion explores the implications of these findings for the trawl-based harvest of snapper.

8.2 The swimming capacities of snapper

Throughout this thesis, critical swimming speed, U_{crit}, was used as a measure of swimming capacity. Although not a true indicator of maximum sustainable velocity, since the test culminates with the recruitment and fatigue of the white muscle (WM), the U_{crit} test nonetheless provided a straightforward and experimentally repeatable (i.e. Chapter 4) means of estimating the swimming performance of snapper. Snapper exhibited a relatively high swimming capacity, with critical velocities of up to 7.1 bl s^{-1} attained. Swimming performance was, as expected, highly dependent on body size, with swimming velocities

increasing (in absolute terms) with increasing fish size, while relative performance favoured smaller fish. The scaling of critical swimming speed was defined by the function

$$U_{crit}\ (m\ s^{-1}) = 0.003142(length) + 0.2669$$

where length is in mm.

Swimming performance also exhibited considerable variation in response to environmental conditions. Critical swimming speed was sensitive to changes in acclimation temperature, with thermal performance curves evident over the temperature range investigated. Despite the limited resolution of these curves, swimming performance appeared to be optimal at temperatures between 18 and 24 ^{o}C, with the suggestion that within this range, optimal temperatures might be slightly higher for larger snapper. In contrast, acclimation to 12 ^{o}C resulted in U_{crit} being reduced between 17 and 27%, relative to fish of comparable size acclimated to 18^{o}C. In addition, the swimming performance of snapper was influenced by ambient oxygen availability, with U_{crit} significantly reduced at oxygen tensions below 80 mmHg.

8.2.1 Implications of swimming performance for capture

The importance of swimming performance with regards to trawl-based capture fisheries has been described by Wardle (1993), He (1993) and Winger et al. (1999; 2010). Once herded by the sweeps into the path of the net, fish attempt to maintain pace with the approaching trawl; their ability to do so is ultimately determined by their capacity for sustained swimming relative to the velocity of the net. Where the latter exceeds the capabilities of the fish, the recruitment of the WM and burst swimming behaviours culminates in fatigue, with the fish swept back into the codend. Once within the codend, fish frequently experience – in addition to the preceding metabolic exhaustion – impact injuries as they collide with the net, debris or other fish, and crush injury and asphyxia as they become impinged against the net or accumulating catch. Consequently, fish that are retained within the codend for some time are often dead or dying when the catch is landed.

Within the present study, the size of snapper for which the critical swimming speeds could be determined was limited by size and velocity constraints of the swimming flume; estimation of the critical swimming speeds of snapper of commercially-relevant size

therefore requires extrapolation from the present data. Data describing the length and age of snapper at landing within the SNA1 trawl fishery indicates a mean catch size of 30-35 cm in length, with a total range of approximately 20 to 70 cm (Davies and Walsh, 1995). Based on the allometric equations derived in Chapter 3, critical swimming speeds for 30-35 cm snapper are estimated at 1.2 to 1.4 m s^{-1}. Since the average tow speed during the harvest of snapper is 2.5-3.5 knots (1.3-1.8 m s^{-1}), the effort required by these fish to maintain their position within the net likely approaches or exceeds their physiological limits. Considering that the duration of these trawls is often 2-3 hours, it is likely fish will ultimately become fatigued, and hence become part of the accumulating catch. In addition, while the current data suggest a swimming capacity of 2.5 m s^{-1} for snapper 70 cm in length, estimates of the critical swimming speeds of 20 and 25 cm snapper (25 cm being the minimum legal size) are 0.9 and 1.1 m s^{-1}. The data therefore suggests that the swimming capacity of smaller snapper – even those of legal size – will be exceeded by even the lowest of commonly used tow speeds. In addition to the implications that the resulting fatigue will have for the quality of small but legally sized fish (see Section 8.3.1), there are likely significant implications for the survival of fish of sub-legal size. In particular, fatigue may constrain the abilities of juvenile snapper to exploit bycatch-reduction devices (BRDs), many of which require deliberate and directed swimming efforts to negotiate (Breen et al., 2004; Suuronen, 2005). Should these fish be retained within the net, they will rapidly fatigue, ultimately suffering mortality resulting from asphyxia and crush injury.

It is of note, however, that tow speeds may not accurately reflect the velocity of water flow through the net. Entrainment of water by the meshes of the net may reduce flow velocities within the codend to as little as 30% of the tow speed, although the degree of entrainment varies significantly with differences in net design, mesh size and geometry, and the volume of catch within the codend (Ferro, 1998; Thiele et al., 1997; Meyler, 2008). We are unaware of any data detailing how entrainment might alter water velocities within the nets typically used during snapper trawls; however, it would seem that this information represents an important link in correlating harvest conditions with the physiology of landed fish, since it is the velocities inside the net which will ultimately determine the behaviour and abilities of the fish, and hence the nature and severity of stress and injury experienced.

In addition to the variation in swimming performance resulting from differences in body size, the studies within this thesis highlight the need to consider environmental factors when

relating swimming capacity and harvesting conditions. In particular, geographic and seasonal variation in water temperature may be associated with considerable variation in swimming capacity, as might water quality. With regards to the latter, naturally occurring hypoxia of the severity required to significantly impact swimming performance is unlikely to be a significant concern in coastal and offshore areas (i.e. Patel, 2011); however, Davis (2002) suggested that fish may experience hypoxia within the codend of a trawl, resulting from the accumulation of fish. We are aware of no measurements that suggest the extent to which any hypoxia may develop.

8.3 Consequences of exhaustive exercise and trawl capture for tissue biochemistry

During exhaustive exercise, snapper incur significant metabolic, acid-base and haematological perturbations, similar to those observed in other strong swimming species (especially salmonids; Wood et al., 1983; Milligan and Wood, 1986; Pagnotta and Milligan, 1991; Schulte et al., 1992; Milligan and Girard, 1993; Pagnotta et al., 1994; Wang et al., 1994*a*; Richards et al., 2002*a*). Due to the reservations regarding the accuracy of the WM metabolite status documented in Chapter 5, it is difficult to be sure as to the exact magnitude of the disturbance. However, the use of plasma lactate and pH as a proxy for muscle lactate and pH suggests that the lactacidosis is significant, both in size (with plasma lactate reaching 15 mmol l^{-1}) and duration (requiring 6 hour for the restoration of metabolic status). The residual elevation of $\dot{M}O_2$ post-exercise, described as the excess post-exercise oxygen consumption (EPOC), is further indicative of a significant metabolic disturbance within the muscle, since EPOC is attributed to the aerobic cost of reinstating metabolic, acid-base and ionic homeostasis following an exhaustive event (Wood, 1991; Scarabello et al., 1991, 1992). There was also evidence of a considerable stress response, by way of a sharp rise in cortisol over the first hour post-exercise.

Snapper caught during a trawl survey operated in similar manner to that of a commercial trawl also exhibited significant stress and physical exhaustion as a result of capture. While qualitatively similar to that of the response induced by exhaustive exercise within the laboratory, the magnitude of the perturbations in metabolic and acid-base status were greater, and recovery protracted, in trawl-caught fish. Further, WM lactate concentrations in trawl

caught fish were amongst the highest reported within the literature for laboratory- or field-based studies, with an estimated 95% depletion of glycogen, similar to that seen in trawl-caught Atlantic cod (Fraser et al., 1965). It is unclear whether the differences in the magnitude of the response between the two studies reflect the greater severity of capture stresses relative to those in the laboratory, or whether they reflect size and temperature differences, both of which may reflect the magnitude of the anaerobic load within the WM (Dalla Via et al., 1989; Ferguson et al., 1993; Kieffer et al., 1994, 1996; Sfakianakis and Kentouri, 2010); in either case, the observations of mortality in trawl-caught but not laboratory-exercised fish suggest a greater overall stress response induced in trawl-caught fish.

8.3.1 Implications for product quality

Post-mortem, the viability of tissues depends on their ability to continue to produce ATP for the maintenance of homeostasis; the depletion of energy reserves associated with peri-mortem stress and/or fatigue is therefore associated with the rapid onset and progression of degradative processes. Although the measurement of quality indicator profiles post-mortem was outside the scope of the current study, it is assumed that the degree of metabolic disturbance observed following both laboratory and field-based studies indicates an energy deplete state within the WM, which would result in the rapid deterioration of product quality post-harvest. For example, Lowe et al. (1993) documented that rested snapper exhibited a longer pre-rigor period and maintained lower K-values than did snapper caught by longline or exercised to exhaustion within the laboratory. Further, the onset and rate of development of rigor was correlated with the plasma lactate concentration at the time of harvest. That peak lactate levels observed during the present studies were more than double those observed by Lowe et al. (1993), with muscle lactate concentrations also being higher, suggests an even more rapid progression of autolysis and loss of quality attributes in trawl caught fish. This conclusion would be consistent with the observation in other fisheries that trawl-caught fish are typically of poorer quality than are fish caught by other capture methods (Fraser et al., 1965; Turunen et al., 1994; Bjordal, 2002; Rotabakk et al., 2011). The reduced quality of snapper caught by trawl would presumably preclude it from use in high-value products.

8.4 The recovery potential of snapper following exhaustive exercise.

Following exhaustion induced by a laboratory-based incremental exercise test, snapper exhibit a rapid recovery, with disturbances in metabolic and acid-base status corrected within 6 hours. In contrast, snapper surviving capture during trawl surveys demonstrate only partial recovery over an 18 hour period, with plasma and muscle pH and lactate apparently returning to levels consistent with rested fish, while other perturbations, notably the depletion of muscle and liver glycogen, show no signs of recovery, and cortisol remains elevated. These results suggest that while snapper have the capacity to recovery rapidly from exhaustive exercise, the multiple stressors associated with commercial harvest incite a disturbance requiring an extended recovery period. It should also be noted that the conditions in which fish are held post-capture may influence recovery dynamics; chronic stress associated with the retention of wild-caught fish has been implicated in the impairment of recovery (Ellis, 1964; Parker et al., 2003), as conditions may differ significantly from the natural environment with respect to light intensity, temperature, pressure and density of individuals (Broadhurst et al., 2006). It may therefore be important to attempt to characterise the conditions that are most stressful to snapper, and therefore most likely to impede recovery, so that attempts at mitigation may occur. The present study suggests that hypoxia – which one might expect to develop within holding systems as a result of the density of fish retained – is not a significant hindrance to metabolic recovery, where oxygen tensions are maintained above 60-80 mmHg.

8.5 Consequences of trawl capture for sustainability

High rates of incidental capture of juvenile and non-target species are often associated with trawl fisheries, and although steps are frequently taken in attempt to reduce the retention of these fish within the net, they seldom preclude fish from first interacting with the fishing gears and the stresses associated with capture (Chopin and Arimoto, 1995; Suuronen, 2005). Further, the ability of fish to utilise exclusion and bycatch-reduction devices is a function of their swimming capacity relative to the water velocities inside the net (Breen et al., 2004; Suuronen, 2005; Winger et al., 2010). Disproportionately high tow speeds may compromise the ability of fish to escape, resulting in high rates of juvenile capture. Where high towing

velocities are required, for example to ensure the capture of larger fish, the design of BRDs should account for the potentially reduced swimming performance of juvenile fish.

The interaction of fish with fishing gears is frequently associated with mortality, as a direct result of severe physiological disturbance and/or physical injury sustained during capture, and may be compounded by indirect mortality resulting from increased predation or infection of wounds. An understanding of discard and escapee mortality is imperative for the accurate management of fish stocks, since insufficient allowance for capture-related mortality underestimates the biomass lost as a result of fishing activities. (Davis, 2002; Diamond and Campbell, 2009; Frick et al., 2010). In Chapter 6, trawl-caught snapper were observed to have mortality rates of 14%; however, as outlined in the associated discussion, this likely underestimates the true mortality associated with the discard of juvenile snapper as bycatch. In Chapter 4, critical swimming performance was reduced 30% following an exhaustive event. Whether the extent or duration of impairment in swimming performance is greater in a commercial setting is unknown, since there is greater potential for skin or fin damage; however, this highlights the potential for mortality arising from impaired swimming – particularly escape – abilities. The Ministry for Primary Industries currently estimates that for snapper, the rate of incidental mortality associated with trawl fisheries is approximately 11% (Ministry for Primary Industries, 2013*a*). In light of the estimates determined during the present study, and further, those of Sumpton and Jackson (2005) and Stewart (2008), attempts should be made to further investigate discard mortality in snapper, and to reconcile these rates with management allowances, such that the latter accurately reflect the cost of fishing activities to the stock.

8.6 Application of thesis findings

The studies contained within this thesis have demonstrated the swimming capacity of snapper, and that current commercial trawling practices likely exceed this capacity, with consequences for both the quality of retained catch and for the survival of juvenile fish. Swimming capacity is a key determinant of the nature and magnitude of stress experienced during capture; as such, consideration of these capacities may present a means of refining

harvesting protocols or technologies to reduce the severity of capture-related stresses and the associated negative outcomes. For example, reducing the velocity at which water passes through the trawl net to speeds sustainable by smaller classes of fish may permit the landing of fish in better condition, or for higher rates of survival in escaping and discarded juvenile fish. The specific water velocities required for such an outcome will likely vary with season and geographic location, since water temperature exerts a strong influence on swimming performance. In addition, the size distribution of the fish likely to be caught will likely be an important consideration. For example, in areas where higher proportions of smaller snapper are caught, it might be beneficial to implement lower velocities that better account for the lower swimming capacities of these fish. Where the average catch size is somewhat larger, higher velocities might be appropriate, to ensure they are sufficient to permit the capture of the larger, faster fish.

While the specifics of how water velocities may be reduced are beyond the scope of this thesis, they may be as simple as reducing tow speed, possibly in combination with reduced tow duration, in order to minimise the impact of crush injuries. Alternatively, it might be possible to use net deign to manipulate water entrainment, creating "zones" of differing water velocities and providing low-velocity "refuges" for smaller fish. The strategic placement of low-velocity zones might also enhance the functionality of BRDs. The use of net design to create varying water velocity profiles through the net may avoid the need to reduce overall tow speed, which may compromise the ability to catch larger snapper.

In addition to attempts at mitigating the stresses associated with capture, the present study also demonstrates that it may be possible to exploit the recovery potential of snapper as a means of improving product quality. For example, the trawl surveys carried out during this study demonstrate that a proportion of the catch is landed alive, and that not only are many of these fish able to be kept alive post-capture, they exhibit signs of metabolic recovery. Since fish yield the highest quality product when harvested under rested conditions, it may be possible to allow snapper to recover from capture stresses in some form of on-board holding tank(s), prior to slaughter and/or provision to live-fish markets.

8.7 Future directions

The physiological response of fish to capture by commercial fishing gears is a function of their interaction with the gear, and varies in response to different biological, environmental and operational factors (Davis, 2002; Ryer, 2004; Suuronen, 2005; Broadhurst et al., 2006). A comprehensive understanding of the consequences of capture for physiological condition, mortality and product quality therefore requires that the response to capture be determined across a range of possible fishing conditions. For example, exposure to warmer temperatures may exacerbate capture stresses, resulting in increased mortality and behavioural impairments (Turunen et al., 1994; Olla et al., 1998; Davis and Olla, 2001; Davis et al., 2001; Davis and Schreck, 2005; Meka and McCormick, 2005; Suuronen 2005; Gale et al., 2011). However, since reduced water temperatures are associated with lower swimming speeds – a reduction of up to 27% was observed for snapper acclimated to 12 ^{o}C compared with those acclimated to 18 ^{o}C – fish may be more susceptible to fatigue or collision with other fish and/or the nets under cooler conditions (Gale et al., 2013).

Multiple aspects of capture and handling processes are implicated in elevated capture stress and mortality, including tow duration (Oddson et al., 1994; Hatulla et al., 1995; Olla et al., 1997; Frick et al., 2010) and tow speed (Olla et al., 1997). Indeed, subtle differences in tow duration, and consequently in catch size may explain the differences in the lethargy and mortality of fish between the two trawls in Chapter 6. In addition, larger catch size is associated with an increased sorting period, and hence exposure to air, which further exacerbates physiological stress and mortality (Davis and Olla, 2002; Davis, 2005; Davis and Schreck, 2005; Sumpton and Jackson, 2005; Frick et al., 2010). It may therefore be of value to further investigate the effects of tow velocity and duration of the condition of snapper at landing, and of the implications for the rates of capture and survival of juvenile snapper. In addition, the determination of water velocity profiles within a generic snapper trawl net (should they differ from the tow speed) would greatly aid in understanding the relationship between tow speed and the physiological response to capture, and the possible use of net design to reduce capture stress.

In addition to understanding the physiological consequences of commercial harvest, there exists room for improvement in the understanding of the recovery potential of

snapper following capture. As snapper appear capable of a degree of metabolic recovery following capture, there exists the potential for the development of a system that permits recovery prior to a rested-type harvest. This would, however, require an investigation of the conditions that encourage recovery and minimise any stress associated with retention. In particular, the potential value of low-velocity exercise as a means of expediting metabolic recovery should be investigated. The concept of retention for recovery could possibly be extended to juvenile snapper, as Farrell et al. (2001a, 2001b) highlight the potential of the Fraser box to encourage survival and expedite metabolic recovery in salmon prior to release. A more complete understanding of mortality rates in discarded juvenile snapper would be required in order to understand the potential benefits such a system may offer.

Field studies in which the capture methods mimic those used in commercial operations are likely the most appropriate for the determination of the physiological response to capture; however, in addition to prohibitive financial and logistical constraints, they are associated with a lack of control over environmental and experimental conditions and a lack of appropriate controls, which together preclude the determination of the importance of various stressors to the development of the overall stress response (Davis, 2002; Broadhurst et al., 2006). In contrast, laboratory-based simulations, whilst criticised for their lack of realism and inability to simulate certain stressors (i.e. crush injury), do afford the control of experimental conditions and opportunity for replication necessary to investigate the physiological response to specific stressors or combinations of stressors (Davis, 2002; Broadhurst et al., 2006). A combination of field- and laboratory-based studies may therefore be the most productive way in which to resolve the influence of specific stressors or conditions, determining the basic response or potential for interaction with the laboratory, and validating the observations in the field (Davis, 2002).

REFERENCES

Agutter, P.S. and Wheatley, D.N. (2004). Metabolic scaling: consensus or controversy? *Theoretical Biology and Medical Modelling* **1**, 13-23

Almeida-Val, V.M.F., Val, A.L., Duncan, W.P., Souza, F.C.A., Paula-Silva, M.N. and Land, S. (2000). Scaling effects on hypoxia tolerance in the Amazon fish *Astronotus ocellatus* (Perciformes: Cichlidae): contribution of tissue enzyme levels. *Comparative Biochemistry and Physiology Part B* **125**, 219-226

Alsop, D.H. and Wood, C.M. (1997). The interactive effects of feeding and exercise on oxygen consumption, swimming performance and protein usage in juvenile rainbow trout (*Oncorhynchus mykiss*). *Journal of Experimental Biology* **200**, 2337-2346

Altringham, J.D. and Johnston, I.A. (1985). Effects of phosphate on the contractile properties of fast and slow muscle fibres from an Antarctic fish. *Journal of Physiology* **368**, 491-500

Altringham, J.D. and Ellerby, D.J. (1999). Fish swimming: patterns in muscle function. *Journal of Experimental Biology* **202**, 3397-3403

Alverson, D.L., Freeberg, M.A., Murawski, S.A. and Pope, J.G. (1994). A Global Assessment of Fisheries Bycatch and Discards. FAO Fisheries Technical Paper, No. 339. Food and Agriculture Organisation of the United Nations, Rome. 233p.

Armstong, J.D., Priede, I.G. and Lucus, M.C. (1992). The link between respiratory capacity and changing metabolic demands during growth of northern pike, *Esox lucius* L. *Journal of Fish Biology* **41** (Supplement B), 65-75

Bainbridge, R. (1958). The speed and swimming of fish as related to the frequency and the amplitude of the tail beat. *Journal of Experimental Biology* **35**, 109-133

Bainbridge, R. (1960). Speed and stamina in three fish. *Journal of Experimental Biology* **37**, 129-153

Bainbridge, R. (1962). Training, speed and stamina in trout. *Journal of Experimental Biology* **39**, 537-555

Bárány, M. (1967). ATPase activity of myosin correlated with speed of muscle shortening. *Journal of General Physiology* **50,** 197-218

Barton, B.A. (1997). Stress in finfish: past, present and future – a historical perspective. In *Fish Stress and Health in Aquaculture*. Society for Experimental Biology Seminar Series, 62. (eds. Iwama, G.K., Pickering, A.D. Sumpter, J.P. and Schreck, C.B.). Cambridge University Press, Cambridge. pp. 1-33

Barton, B.A. and Iwama, G.K. (1991). Physiological changes in fish from stress in aquaculture with emphasis on the response and effects corticosteroids. *Annual Review of Fish Diseases* **1**, 3-26

Barton, B.A., Schreck, C.B. and Sigismondi, L.A. (1986). Multiple acute disturbances evoke cumulative physiological stress responses in juvenile Chinook salmon. *Transactions of the American Fisheries Society* **115**, 245-251

Barton, B.A., Schreck, C.B. and Barton, L.D. (1987). Effects of chronic cortisol administration and daily acute stress on growth, physiological conditions and stress responses in juvenile rainbow trout. *Diseases of Aquatic Organisms* **2**, 173-185

Batty, R.S. and Wardle, C.S. (1979). Restoration of glycogen from lactic acid in the anaerobic swimming muscle of plaice, *Pleuronectes platessa* L. *Journal of Fish Biology* **15**, 509-519

Beamish, F.W.H. (1964*a*). Respiration of fishes with special emphasis on standard oxygen consumption. II. Influence of weight and temperature on respiration of several species. *Canadian Journal of Zoology* **42**, 177-188

Beamish, F.W.H. (1964*b*). Respiration of fishes with special emphasis on standard oxygen consumption. III. Influence of oxygen. *Canadian Journal of Zoology* **42**, 355-366

Beamish, F.W.H. (1966). Muscular fatigue and mortality in haddock, *Melanogrammus aeglefinus*, caught by otter trawl. *Journal of the Fisheries Research Board of Canada* **23**, 1507-1521

Beamish, F.W.H. (1978). Swimming capacity. In *Fish Physiology. Vol. 7. Locomotion* (eds. Hoar, W.S. and Randall, D.J.). Academic Press, New York. pp. 101-187

Beamish, F.W.H. and Mookherjii, P.S. (1964). Respiration of fishes with special emphasis on standard oxygen consumption. I. Influence of weight and temperature on respiration of goldfish, *Carassius auratus* L. *Canadian Journal of Zoology* **42**, 161-175

Berg, T., Erickson, U. and Nordtvedt, T.S. (1997). Rigor mortis of Atlantic salmon (*Salmo salar*) and effects of stress. *Journal of Food Science* **62**, 439-446

Bennett, A.F. (1990). Thermal dependence of locomotor capacity. *American Journal of Physiology* **259**, R253-R258

Berschick, P., Bridges, C.R. and Grieshaber, M.K. (1987). The influences of hyperoxia, hypoxia and temperature on the respiratory physiology of the intertidal rockpool fish *Gobius cobitis* Pallas. *Journal of Experimental Biology* **130**, 369-387

Bjordal, A. (2002). The use of technical measures in responsible fisheries: regulations of fishing gear. In *A Fishery Manager's Guidebook: Management Measures and Their Application*. (ed. Cochrane, K.L). FAO Fisheries Technical Paper No. 424. Food and Agriculture Organisation of the United Nations, Rome. 231p.

Black, S.E. (2002). Extension of cell viability in rested post-mortem teleost white muscle. PhD thesis, University of Canterbury, Christchurch. 311p.

Black, S.E., Jerrett, A.R. and Forster, M.E. (2004). Extension of the pre-rigor period in ischemic white muscle from yellow-eye mullet (*Aldrichetta forsteri*) and New Zealand snapper (*Pagrus auratus*) as affected by hyperbaric oxygen treatment. *Journal of Food Science* **69**, 297-302

Blaikie, H.B. and Kerr, S.R. (1996). Effect of activity level on the apparent heat increment in Atlantic cod, *Gadus morhua*. *Canadian Journal of Fisheries and Aquatic Sciences* **53**, 2093-2099

Blaxter, J.H.S. and Dickson, W. (1959). Observations on the swimming speeds of fish. *Journal Conseil International pour l'Exploration de la Mer* **24**, 472-479

Boesgaard, L., Nielsen, M.E. and Rosenkilde, P. (1993). Moderate exercise decreases plasma cortisol levels in Atlantic salmon (*Salmo salar*). *Comparative Biochemistry and Physiology* **106A**, 641-643

Bokma, F. (2004). Evidence against a universal metabolic allometry. *Functional Ecology* **18**, 184-187

Boldsen, M.M., Norin, T. and Malte, H. (2013). Temporal repeatability of metabolic rate and the effect of organ mass and enzyme activity on metabolism in European eel (*Anguilla anguilla*). *Comparative Biochemistry and Physiology Part A* **165**, 22-29

Bone, Q. (1978). Locomotor muscle. In *Fish Physiology. Vol. 7. Locomotion.* (eds. Hoar, W.S. and Randall, D.J.). Academic Press, New York. pp. 361-424

Bone, Q., Kiceniuk, J. and Jones, D.R. (1978). On the role of the different fibre types in fish myotomes at intermediate swimming speeds. *Fisheries Bulletin* **76**, 691-699

Bone, Q., Marshall, N.B. and Blaxter, J.H.S. (1995). *Biology of Fishes*. 2nd edition. Chapman and Hall, London. 332p.

Booth, J.H. (1978). The distribution of blood flow in the gills of fish: application of a new technique to rainbow trout (*Salmo gairdneri*). *Journal of Experimental Biology* **73**, 11-129

Booth, J.H. (1979). The effects of oxygen supply, epinephrine and acetylcholine on the distribution of blood flow in trout gills. *Journal of Experimental Biology* **83**, 31-39

Borch, K., Jensen, F.B. and Andersen, B.B. (1993). Cardiac activity, ventilation rate and acid-base regulation in rainbow trout exposed to hypoxia and combined hypoxia and hypercapnia. *Fish Physiology and Biochemistry* **12**, 101-110

Bosworth, B.G., Small, B.C., Gregory, D., Kim, J., Black, S. and Jerrett, A. (2007). Effects of rested-harvest using the anaesthetic AQUI-S^{TM} on channel catfish, *Ictalurus punctatus*, physiology and fillet quality. *Aquaculture* **262**, 302-318.

Botsford L.W., Castilla J.C., and Peterson C.H. (1997). The management of fisheries and marine ecosystems. *Science* **277**, 509-515

Boutilier, R.G. (2001). Mechanisms of cell survival in hypoxia and hypothermia. *Journal of Experimental Biology* **204**, 3171-3181

Brauner, C.J., Iwama, G.K. and Randall, D.J. (1994). The effect of short-duration seawater exposure on the swimming performance of wild and hatchery-reared juvenile coho salmon (*Oncorhynchus kisutch*) during smoltification. *Canadian Journal of Fisheries and Aquatic Sciences* **51**, 2188-2194

Breen, M., Dyson, J., O'Neill, F.G., Jones, E. and Haigh, M. (2004). Swimming endurance of haddock (*Melanogrammus aeglefinus* L.) at prolonged and sustained swimming speeds and its role in their capture by towed fishing gears. *ICES Journal of Marine Sciences* **61**, 1071-1079

Brett, J.R. (1964). The respiratory metabolism and swimming performance of young sockeye salmon. *Journal of the Fisheries Research Board of Canada* **21**, 1183-1226

Brett, J.R. (1965). The relation of size to rate of oxygen consumption and sustained swimming speed of sockeye salmon (*Oncorhynchus nerka*). *Journal of the Fisheries Research Board of Canada* **22**, 1491-1501

Brett, J.R. (1967). Swimming performance of sockeye salmon (*Oncorhynchus nerka*) in relation to fatigue time and temperature. *Journal of the Fisheries Research Board of Canada* **24**, 1731-1740

Brett, J.R. (1971). Energetic responses of salmon to temperature. A study of some thermal relations in the physiology and freshwater ecology of sockeye salmon (*Oncorhynchus nerka*). *American Zoologist* **11**, 99-113

Brett, J.R. and Glass, N.R. (1973). Metabolic rates and critical swimming speeds of sockeye salmon (*Oncorhynchus nerka*) in relation to size and temperature. *Journal of the Fisheries Research Board of Canada* **30**, 379-387

Brett, J.R. and Groves, T.D.D. (1978). Physiological Energetics. In *Fish Physiology. Vol. 8 Bioenegetics and Growth*. (eds. Hoar, W.S., Randall, D.J. and Brett, J.R.). pp. 279-352. Academic Press, New York.

Broadhurst, M.K., Suuronen, P. and Hulme, A. (2006). Estimating collateral mortality from towed fishing gears. *Fish and Fisheries* **7**, 180-218

Brooks, J.R., Griffin, V.K. and Kattan, M.W. (1986). A modified method for total carbohydrate analysis of glucose syrups, maltodextrins and other starch hydrolysis products. *Cereal Chemistry* **63**, 465-466

Burgetz, I.J., Rojas-Vargas, A., Hinch, S.G. and Randall, D.J. (1998). Initial recruitment of anaerobic metabolism during sub-maximal swimming in rainbow trout (*Oncorhynchus mykiss*). *Journal of Experimental Biology* **201**, 2711-2721

Burleson, M.L. (2009). Sensory innervations of the gills: O_2-sensitive chemoreceptors and mechanoreceptors. *Acta Histochemica* **111**, 196-206

Burton, T., Killen, S.S., Armstrong, J.D. and Metcalfe, N.B. (2011). What causes intraspecific variation in resting metabolic rate and what are its ecological consequences? *Proceedings of the Royal Society B* **278**, 3465-3473

Bushnell, P.G. (1984). Oxygen consumption and swimming performance in hypoxia-acclimated rainbow trout *Salmo gairdneri*. *Journal of Experimental Biology* **113**, 225-235

Caldwell, C.A., Kattesh, H.G. and Strange, R.J. (1991). Distribution of cortisol among free and protein-bound fractions in rainbow trout (*Oncorhynchus mykiss*): evidence of control by sexual maturation. *Comparative Biochemistry and Physiology* **99A**, 593-595

Campbell, N.A. and Reece, J.B. (2005). *Biology*. 7th edition. Pearson/Benjamin Cummings, San Francisco.1231p.

Carroll, N.V., Longley, R.W. and Roe, J.H. (1956). The determination of glycogen in liver and muscle by use of anthrone reagent. *Journal of Biological Chemistry* **220**, 583-593

Cech, J.J. (1990). Respirometry. In *Methods for Fish Biology*. (eds. Schreck, C.B. and Moyle, P.B.). American Fisheries Society, Bethesda. pp. 335-362

Cech, J.J. and Crocker, C.E. (2002). Physiology of sturgeon: effects of hypoxia and hypercapnia. *Journal of Applied Ichthyology* **18**, 320-324

Cerezo, J. and Garcia, B.G. (2004). The effects of oxygen levels on oxygen consumption, survival and ventilatory frequency of sharpsnout sea bream (*Diplodus puntazzo* Gmelin, 1789) at different conditions of temperature and fish weight. *Journal of Applied Icthyology* **20**, 488-492

Chabot, D. and Claireaux, G. (2008). Environmental hypoxia as a metabolic constraint on fish: the case of Atlantic cod, *Gadus morhua*. *Marine Pollution Bulletin* **57**, 287-294

Champe, P.C., Harvey, R.A. and Ferrier, D.R. (2008). Bioenergetics and oxidative phosphorylation. In *Lippincott's Illustrated Reviews: Biochemistry* (eds. Champe, P.C. and Harvey, R.A.). J.B. Lippincott, Philadelphia. 443p.

Chapman, L.J. and McKenzie, D.J. (2009). Behavioural responses and ecological consequences. In *Fish Physiology, Vol. 27. Hypoxia*. (eds. Richards, J.G., Farrell, A.P. and Brauner, C.J.). pp. 25-77. Elsevier, London.

Choi, D., Cole, K.J., Goodpaster, B.H., Fink, W.J. and Costill, D.L. (1994). Effect of passive and active recovery on the resynthesis of muscle glycogen. *Medical Science and Sports Exercise* **26**, 992-996

Chopin, F.S. and Arimoto, T. (1995). The condition of fish escaping from fishing gears – a review. *Fisheries Research* **21**, 315-327

Claireaux, G. and Lagardère, J.P. (1999). Influence of temperature, oxygen and salinity on the metabolism of the European sea bass. *Journal of Sea Research* **42**, 157-168

Claireaux, G., Webber, D.M., Lagardère, J.P. and Kerr, S.P. (2000). Influence of water temperature and oxygenation on the aerobic metabolic scope of Atlantic cod (*Gadus morhua*). *Journal of Sea Research* **44**, 257-265

Claireaux, G., McKenzie, D.J., Genge, A.G., Chatelier, A., Aubin, J. and Farrell, A.P. (2005). Linking swimming performance, cardiac pumping ability and cardiac anatomy in rainbow trout. *Journal of Experimental Biology* **208**, 1775-1784

Claireaux, G., Couturier, C. and Groison, A.L. (2006). Effect of temperature on maximum swimming speed and cost of transport in juvenile European sea bass (*Dicentrarchus labrax*). *Journal of Experimental Biology* **209**, 3420-3428

Clark, T.D., Sandblom, E. and Jutfelt, F. (2013). Aerobic scope measurements of fishes in an era of climate change: respirometry, relevance and recommendations. *Journal of Experimental Biology* **216**, 2771-2782

Clarke, A. and Johnston, N.M. (1999). Scaling of metabolic rate with body mass and temperature in teleost fish. *Journal of Animal Ecology* **68**, 893-905

Clarke, A. and Fraser, K.P.P. (2004). Why does metabolism scale with temperature? *Functional Ecology* **18**, 243-251

Cook, D.G. and Herbert, N.A. (2012). Low O_2 avoidance is associated with physiological perturbation but not exhaustion in the snapper (*Pagrus auratus*: Sparidae). *Comparative Biochemistry and Physiology Part A* **162**, 310-316

Cook, D.G., Wells, R.M.G. and Herbert, N.A. (2011). Anaemia adjusts the aerobic physiology of snapper (*Pagrus auratus*) and modulates hypoxia avoidance behaviours during oxygen choice presentations. *Journal of Experimental Biology* **214**, 2927-2934

Cook, D.G., Iftikar, F.I., Baker, D.W., Hickey, A.J.R. and Herbert, N.A. (2013). Low-O_2 acclimation shifts the hypoxia avoidance behaviour of snapper (*Pagrus auratus*) with only subtle changes in aerobic and anaerobic function. *Journal of Experimental Biology* **216**, 369-378

Cossins, A.R. and Bowler, K. (1987). *Temperature Biology of Animals*. Chapman Hall, London. 339p.

Cowey, C.B., Higuera, M. and Adron, J.W. (1977). The effect of dietary composition and of insulin on gluconeogenesis in rainbow trout. *British Journal of Nutrition* **38**, 385-395

Cutts, C.J., Metcalfe, N.B. and Taylor, A.C. (1998). Aggression and growth depression in juvenile Atlantic salmon: the consequences of individual variation in metabolic rate. *Journal of Fish Biology* **52**, 1026-1037

Cutts, C.J., Adams, C.E. and Campbell, A. (2001). Stability of physiological and behavioural determinants of performance in Arctic char (*Salvelinus alpinus*). *Canadian Journal of Fisheries and Aquatic Sciences* **58**, 961-968

Cutts, C.J., Metcalfe, N.B. and Taylor, A.C. (2002). Juvenile Atlantic salmon (*Salmo salar*) with relatively high standard metabolic rates have small metabolic scopes. *Functional Ecology* **16**, 73-78

Dalgaard, P. (2002). Modelling and predicting the shelf-life of seafood. In *Safety and Quality Issues in Fish Processing*. (ed. Bremner, H.A.). Woodhead Publishing Limited, Cambridge. pp. 191-219

Dalla Via, J., Huber, M., Wieser, W. and Lackner, R. (1989). Temperautre-related responses of intermediary metabolism to forced exercise and recovery in juvenile *Rutilus rutilus* (L.)(Cyprinidae: Teleostei). *Physiological Zoology* **62**, 964-976

Davies, N.M. and Walshe, C. (1995). Length and age composition of commercial snapper landings in the Auckland Fishery Management Area, 1988-1994. New Zealand Fisheries Data Report No. 58. MAF Fisheries, Wellington.

Davis, M.W. (2002). Key principles for understanding fish bycatch discard mortality. *Canadian Journal of Fisheries and Aquatic Sciences* **52**, 1834-1843

Davis, M.W. (2005). Behaviour impairment in captured and released sablefish: ecological consequences and possible substitute measures for delayed discard mortality. *Journal of Fish Biology* **66**, 254-265

Davis, M.W. (2007). Simulated fishing experiments for predicting delayed mortality rates using reflex impairment in restrained fish. *ICES Journal of Marine Science* **64**, 1535-1542

Davis, M.W. and Olla, B.L. (2001). Stress and delayed mortality induced in Pacific halibut by exposure to hooking, net towing, elevated seawater temperature and air: implications for management of bycatch. *Journal of Fisheries Management* **21**, 725-732

Davis, M.W. and Olla, B.L. (2002). Mortality of lingcod towed in a net as related to fish length, seawater temperature and air exposure: a laboratory bycatch study. *North American Journal of Fisheries Management* **24**, 518-524

Davis, M.W. and Parker, S.J. (2004). Fish size and exposure to air: potential effects on behavioural impairment and mortality rates in discarded sablefish. *North American Journal of Fisheries Management* **24**, 518-524

Davis, M.W. and Schreck, C.B. (2005). Responses by Pacific halibut to air exposure: lack of correspondence among plasma constituents and mortality. *Transactions of the American Fisheries Society* **134**, 991-998

Davis, M.W., Olla, B.L. and Schreck, C.B. (2001). Stress induced by hooking, net towing, elevated sea water temperature and air in sablefish: lack of concordance between mortality and physiological measures of stress. *Journal of Fish Biology* **58**, 1-15

Davis, M.W. and Ottmar, M.L. (2006). Wounding and reflex impairment may be predictors for mortality in discarded or escaped fish. *Fisheries Research* **82**, 1-6

Davison, W. and Goldspink, G. (1977). The effect of prolonged exercise on the lateral musculature of the brown trout (*Salmo trutta*). *Journal of Experimental Biology* **70**, 1-12

Davison, W., Goldspink, G. and Johnston, I.A. (1976). Division of labour between fish myotomal muscles during swimming. *Journal of Physiology* **263**, 185P-186P

Day, N. and Butler, P.J. (2005). The effects of acclimation to reversed seasonal temperatures on the swimming performance of adult brown trout *Salmo trutta*. *Journal of Experimental Biology* **208**, 2683-2692

Day, R.W. and Quinn, G.P. (1989). Comparisons of treatments after an analysis of variance in ecology. *Ecological Monographs* **59**, 433-463

de Salvo Souza, R.H., Soncini, R., Glass, M.L., Sanches, J.R. and Rantin, F.T. (2001). Ventilation, gill perfusion and blood gases in dourado, *Salminus maxillosus* Valenciennes (Teleostei, Characidae) exposed to graded hypoxia. *Journal of Comparative Physiology B* **171**, 483-489

Diamond, S.L. and Campbell, M.D. (2009). Linking "sink or swim" indicators to delayed mortality in red snapper by using a condition index. *Marine and Coastal Fisheries: Dynamics, Management and Ecosystem Science* **1**, 107-120

Diaz, R.J. (2001). Overview of hypoxia around the world. *Journal of Environmental Quality* **30**, 275-281

Diaz, R.J. and Breitburg, D.L. (2009). The hypoxic environment. In *Fish Physiology, Vol. 27. Hypoxia*. (eds. Richards, J.G., Farrell, A.P. and Brauner, C.J.). Elsevier, London. pp. 1-23.

Dickson, K.A., Donley, J.M., Sepulveda, C. and Bhoopat, L. (2002). Effects of temperature on sustained swimming performance and swimming kinematics of the chub mackerel *Scomber japonicas*. *Journal of Experimental Biology* **205**, 969-980

Dobson, G.P. and Hochachka, P.W. (1987). Role of glycolysis in adenylate depletion and repletion during work and recovery in teleost white muscle. *Journal of Experimental Biology* **129**, 125-140

Dodds, P.S., Rothmann, D.H. and Weitz, J.S. (2001). Re-examination of the "3/4-law" of metabolism. *Journal of Theoretical Biology* **209**, 9-27

Domenici, P., Herbert, N.A., Lefrançois, C., Steffensen, J.F. and McKenzie, D.J. (2013). The effect of hypoxia on fish swimming performance and behaviour. In *Swimming Physiology of Fish: Toward Using Exercise to Farm a Fit Fish in Sustainable Aquaculture* (eds. Palstra, A.P. and Planas, H.V.). Springer-Verlag, Berlin. pp. 129-174.

Dupont-Prinet, A., Claireaux, G. and McKenzie, D.J. (2009). Effects of feeding and hypoxia on cardiac performance and gastrointestinal blood flow during critical speed swimming in the sea bass (*Dicentrarchus labrax*). *Comparative Biochemistry and Physiology* **154A**, 233-230

Dutil, J.D., Sylvester, E.L., Gamache, L., Laroque, R. and Guderley, H. (2007). Burst and coast use, swimming performance and metabolism of Atlantic cod *Gadus morhua* in sub-lethal hypoxic conditions. *Journal of Fish Biology* **71**, 363-375

Dyson, R.D., Cardenas, J.M. and Barsotti, R.J. (1975). The reversibility of skeletal muscle pyruvate kinase and an assessment of its capacity to support glyconeogenesis. *Journal of Biological Chemistry* **250**, 3316-3321.

Ellis, R.J. (1964). The effect of confinement on blood lactate levels in Chinook and coho salmon. *Fish Commission of Oregon: Research Briefs* **10**, 28-34

Erikson, U., Beyer, A.R. and Sigholt, T. (1997). Muscle high-energy phosphates and stress affect K-values during ice storage of Atlantic salmon (*Salmo salar*). *Journal of Food Science* **62**, 43-47

Eros, S. and Milligan, C.L. (1996). The effect of cortisol on recovery from exhaustive exercise in rainbow trout: potential mechanisms of action. *Physiological Zoology* **69**, 1196-1214

Everett, M.V. and Crawford, D.L. (2010). Adaptation versus allometry: population and body mass effects on hypoxic metabolism in *Fundulus grandis*. *Physiological and Biochemical Zoology* **83**, 182-190

Fänge, R. and Nilsson, S. (1985). The fish spleen: structure and function. *Experientia* **41**, 152-158

FAO. (2009). The State of World Fisheries and Aquaculture 2008. Food and Agriculture Organisation of the United Nations, Fisheries and Aquaculture Department, Rome, Italy. 176p.

Farrell, A.P. (1985). A protective effect of adrenalin on the acidotic teleost heart. *Journal of Experimental Biology* **116**, 503-508

Farrell, A.P. (2002). Cardiorespiratory performance in salmonids during exercise at high temperatures: insights into cardiovascular design limitations in fishes. *Comparative Biochemistry and Physiology Part A* **132**, 797-810

Farrell, A.P. (2007*a*). Tribute to P. L. Lutz: a message from the heart – why hypoxic bradycardia in fishes? *Journal of Experimental Biology* **210**, 1715-1725

Farrell, A.P. (2007*b*). Cardiorespiratory performance during prolonged swimming tests with salmonids: a perspective on temperature effects and potential analytical pitfalls. *Philosophical Transactions of the Royal Society B* **362**, 2017-2030

Farrell, A.P. and Clutterham, S.M. (2003). Online venous tensions in rainbow trout during graded exercise at two acclimation temperatures. *Journal of Experimental Biology* **206**, 487-496

Farrell, A.P. and Richards, J.G. (2009). Defining hypoxia: an integrative synthesis of the response of fish to hypoxia. In *Fish Physiology, Vol. 27. Hypoxia*.. (eds. Richards, J.G., Farrell, A.P. and Brauner, C.J.). pp. 487-503. Elsevier, London.

Farrell, A.P. and Steffensen, J.F. (1987). Coronary ligation reduces maximum sustained swimming speed in Chinook salmon *Oncorhynchus tshawytscha. Comparative Biochemistry and Physiology* **87A**, 35-37

Farrell, A.P., Gamperl, A.K. and Birtwell, I.K. (1998). Prolonged swimming, recovery and repeat swimming performance of mature sockeye salmon *Oncorhynchus nerka* exposed to moderate hypoxia and pentachlorophenol. *Journal of Experimental Biology* **201**, 2183-2193

Farrell, A.P., Gallaugher, P., Clarke, C., DeLury, N., Kreiberg, H., Parkhouse, W. and Routledge, R. (2000). Physiological status of coho salmon (*Oncorhynchus kisutch*) captured in commercial nonretention fisheries. *Canadian Journal of Fisheries and Aquatic Sciences* **57**, 1668-1678

Farrell, A.P., Gallaugher, P.E., Fraser, J., Pike, D., Bowering, P., Hadwin, A.K.M., Parkhouse, W. and Routledge, R. (2001*a*). Successful recovery of the physiological status of coho salmon on board a commercial gillnet vessel by means of a newly designed revival box. *Canadian Journal of Fisheries and Aquatic Sciences* **58**, 1932-1946

Farrell, A.P., Gallaugher, P.E. and Routledge, R. (2001*b*). Rapid recovery of exhaustive adult coho salmon after commercial capture by troll fishing. *Canadian Journal of Fisheries and Aquatic Sciences* **58**, 2319-2324

Farrell, A.P., Lee, C.G., Tierney, K., Hodaly, A., Clutterham, S., Healy, M., Hinch, S. and Lotto, A. (2003). Field-based measurements of oxygen uptake and swimming performance with adult Pacific salmon using a mobile respirometer swim tunnel. *Journal of Fish Biology* **62**, 64-84

Ferguson, R.A., Kieffer, J.D. and Tufts, B.L. (1993). The effects of body size on the acid-base and metabolite status in the white muscle of rainbow trout before and after exhaustive exercise. *Journal of Experimental Biology* **180**, 195-207

Ferro, R.S.T. (1988). Some measurements of flow inside codends and a pelagic net. D.A.F.S. Working Paper No. 10188. Department of Agriculture and Fisheries, Scotland. 6p.

Fletcher, G.C., Corrigan, V.K., Summers, G., Leonard, M.J., Jerrett, A.R. and Black, S.E. (2003). Spoilage of rested harvested King salmon (*Oncorhynchus tshawytscha*). *Journal of Food Science* **68**, 2810-2816

Fitzgibbon, Q.P., Strawbridge, A. and Seymour, R.S. (2007). Metabolic scope, swimming performance and the effects of hypoxia in the mulloway, *Argyrosomus japonicas* (Pisces: Sciaenidae). *Aquaculture* **270**, 358-368

Forest and Bird. (2009). Best Fish Guide 2009-2010: How sustainable is New Zealand seafood? (Ecological Assessments). Royal Forest and Bird Protection Society of New Zealand Inc., Wellington. 154p.

Forgan, L.G. (2009). Influence of oxygen supply on metabolism and energetics in fish muscles. PhD thesis, University of Canterbury, Christchurch. 252p.

Forster, M.E., Frankin, C.E., Taylor, H.H. and Davison, W. (1987). The aerobic scope of an Antarctic fish, *Pagothenia borchgrevinki* and its significant for metabolic cod adaptation. *Polar Biology* **8**, 155-159

Fraser, D.I., Weinstein, H.M. and Dyer, W.J. (1965). Post-mortem glycolytic and associated changes in the muscle of trap- and trawl-caught cod. *Journal of the Fisheries Research Board of Canada* **22**, 83-100

Frick, L.H., Walker, T.I. and Reina, R.D. (2010). Trawl capture of Port Jackson sharks, *Heterodontus portusjacksoni*, and gummy sharks, *Mustelus antarcticus*, in a controlled setting: effects of tow duration, air exposure and crowding. *Fisheries Research* **106**, 344-350

Frolow, J. and Milligan, C.L. (2004). Hormonal regulation of glycogen metabolism in white muscle slices from rainbow trout (*Oncorhynchus mykiss,* Walbum). *American Journal of Physiology* **287**, R1344-R1353

Fry, F.E.J. (1947). Effects of the environment on animal activity. *University of Toronto Biological Series*, **55** 1-62

Fry, F.E.J. (1948). The relationship of temperature to oxygen consumption in goldfish. *Biological Bulletin* **94**, 66-77

Fry, F.E.J. (1978). The effect of environmental factors on the physiology of fish. In *Fish Physiology. Vol. 6. Environmental Relations and Behaviour.* (eds. Hoar, W.S. and Randall, D.J.). Academic Press, New York. pp. 1-98

Fry, F.E.J. and Cox, E.T. (1970). A relation of size to swimming speed in rainbow trout. *Journal of the Fisheries Research Board of Canada* **5**, 976-978

Fu, S.J., Zeng, L.Q., Li, X.., Pang, X., Cao, Z.D., Peng, J.L. and Wang, Y.X. (2009). Effects of meal size on excess post-exercise oxygen consumption in fishes with different locomotor and digestive performance. *Journal of Comparative Physiology B* **179**, 509-517

Fu, S.J., Brauner, C.J., Cao, Z.D., Richards, J.G., Peng, J.L., Dhillon, R. and Wang, Y.X. (2011). The effect of acclimation to hypoxia and sustained exercise on subsequent hypoxia tolerance and swimming performance in goldfish (*Carassius auratus*). *Journal of Experimental Biology* **214**, 2080-2088

Furimsky, M., Cooke, S.J., Suski, C.D., Wang, Y. and Tufts, B.L. (2003). Respiratory and circulatory responses to hypoxia in largemouth bass and smallmouth bass: implications for "live-release" angling tournaments. *Transactions of the American Fisheries Society* **132**, 1065-1075

Gale, M.K., Hinch, S.G., Eliason, E.J., Cooke, S.J. and Patterson, D.A. (2011). Physiological impairment of adult sockeye salmon in fresh water after simulated capture-and-release across a range of temperatures. *Fisheries Research* **112**, 85-95

Galloway, B.J. and Kieffer, J.D. (2003). The effects of an acute temperature change on the metabolic recovery from exhaustive exercise in juvenile Atlantic salmon (*Salmo salar*). *Physiological and Biochemical Zoology* **76**, 652-662

Gamperl. A.K. and Driedzic, W.R. (2009). Cardiovascular function and cardiac metabolism. In *Fish Physiology, Vol. 27. Hypoxia*.. (eds. Richards, J.G., Farrell, A.P. and Brauner, C.J.). pp. 301-360. Elsevier, London.

Garrett, R.H. and Grisham, C.M. (2005). *Biochemistry*. 3rd edition. Thomson Brooks, Belmont. 1087p.

Gingerich, A.J., Phillip, D.P. and Suski, C.D. (2010). Effects of nutritional status on metabolic rate, exercise and recovery in a freshwater fish. *Journal of Comparative Physiology B* **180**, 371-384

Girard, S.S. and Milligan, C.L. (1992). Metabolic fate of blood-borne lactate in winter flounder (*Pseudopleuronectes americanus*) during recovery from exhaustive exercise. *Physiological Zoology* **65**, 1114-1134

Glazier, D.S. (2005). Beyond the "3/4-power law": variation in the intra- and interspecific scaling of metabolic rate in animals. *Biological Reviews* **80**, 611-662

Gleeson, T.T. (1996). Post-exercise lactate metabolism: a comparative review of sites, pathways and regulation. *Annual Reviews in Physiology* **58**, 565-581

Goldspink, G. (1977). Mechanics and energetic of muscle in animals of different sizes, with particular reference to the muscle fibre composition of vertebrate muscle. In *Scale Effects in Animal Locomotion.* (ed. Pedley, T.J.). Academic Press, London. pp. 37-55

Goolish, E.M. (1989). The scaling of aerobic and anaerobic muscle power in rainbow trout (*Salmo gairdneri*). *Journal of Experimental Biology* **147**, 493-505

Goolish, E.M. (1991). Aerobic and anaerobic scaling in fish. *Biological Reviews* **66**, 33-56

Green, H.J. (2004). Membrane excitability, weakness and fatigue. *Canadian Journal of Applied Physiology* **29**, 291-307

Gregory, T.R. and Wood, C.M. (1998). Individual variation and interrelationships between swimming performance, growth rate and feeding in juvenile rainbow trout (*Oncorhynchus mykiss*). *Canadian Journal of Fisheries and Aquatic Sciences* **55**, 1583-1590

Gregory, T.R. and Wood, C.M. (1999). The effects of chronic plasma cortisol elevation on the feeding behaviour, growth, competitive ability and swimming performance of juvenile rainbow trout. *Physiological and Biochemical Zoology* **72**, 286-295

Griffiths, J.S. and Alderice, D.F. (1972). Effects of acclimation and acute temperature experience on the swimming speed of juvenile coho salmon. *Journal of the Fisheries Research Board of Canada* **29**, 251-264

Guderley, H. (2004). Metabolic responses to low temperature in fish muscle. *Biological Reviews* **49**, 409-427

Hammer, C. (1995). Fatigue and exercise tests with fish. *Comparative Biochemistry and Physiology* **112A**, 1-20

Hammill, E., Wilson, R.S. and Johnston, I.A. (2004). Sustained swimming performance and muscle structure are altered by thermal acclimation in male mosquitofish. *Journal of Thermal Biology* **29**, 251-257

Hanna, S.K., Haukenes, A.H., Foy, R.H. and Buck, C.L. (2008*a*). Temperature effects on metabolic rate, swimming performance and condition of Pacific cod *Gadus macrocephalus* Tilesius. *Journal of Fish Biology* **72**, 1068-1078

Hanna, S.K., Haukenes, A.H., Foy, R.H. and Buck, C.L. (2008*b*). Temperature effects on recovery of Pacific cod following exhaustive exercise. Resiliency of Gadid Stocks to Fishing and Climate Change, Alaska Sea Grant College Program, January 2008. pp 239-250

Harley S.J., Millar, R.B. and McArdle, B.H. (2000). Estimating unaccounted fishing mortality using selectivity data: an application in the Hauraki Gulf snapper (*Pagrus auratus*) fishery in New Zealand. *Fisheries Research* **45**, 167-178

Hattula, T., Luoma, T., Kostiainen, R., Poutanen, J., Kallio, M. and Suuronen, P. (1995). Effects of catching method on different quality parameters of Baltic herring (*Clupea harengus* L.). *Fisheries Research* **23**, 209-221

He, P. (1993). Swimming speeds of marine fish in relation to fishing gears. *ICES Marine Science Symposium* **196**, 183-189

Heath, A.G. (1973). Ventilatory response of teleost fish to exercise and thermal stress. *American Zoologist* **13**, 491-503

Heath, A.G. and Hughes, G.M. (1973). Cardiovascular and respiratory changes during heat stress in rainbow trout (*Salmo gairdneri*). *Journal of Experimental Biology* **59**, 323-338

Heinimaa, S. (2003). Juvenile years of Atlantic salmon in the wild and in the hatchery: Ecophysiological differences. In *Acta Univeritatis Ouluensis A, Vol. 407*. University of Oulu, Oulu. 36p.

Herbert, N.A. and Steffensen, J.F. (2005). The response of Atlantic cod, *Gadus morhua*, to progressive hypoxia: fish swimming speed and physiological stress. *Marine Biology* **147**, 1403-1412

Herbert, N.A., Skjæraasen, J.E., Nilsen, T., Salvanes, A.G.V. and Steffensen, J.F. (2011). The hypoxia avoidance behaviour of juvenile Atlantic cod (*Gadus morhua*) depends on the provision and pressure level of an O_2 refuge. *Marine Biology* **158**, 737-746

Hill, R.W., Wyse, G.A. and Anderson, M. (2004). *Animal Physiology*. Sinauer Associates, Sunderland, Massachusetts. 770p.

Hilton, Z., Clements, K.D. and Hickey, A.J.R. (2010). Temperature sensitivity of cardiac mitochondria in intertidal and subtidal triplefin fishes. *Journal of Comparative Physiology B* **180**, 979-990

Hochachka, P.W. (1985). Fuels and pathways as designed systems for support of muscle work. *Journal of Experimental Biology* **115**, 149-164

Hochachka, P.W. and McClelland, G.B. (1997). Cellular metabolic homeostasis during large-scale change in ATP turnover rates in muscles. *Journal of Experimental Biology* **200**, 181-186

Holeton, G.F. and Heisler, N. (1983). Contribution of net ion transfer mechanisms to acid-base regulation after exhausting activity in the larger spotted dogfish (*Scyliorhinus stellaris*). *Journal of Experimental Biology* **103**, 31-46

Holeton, G.F. and Randall, D.J. (1967). The effect of hypoxia upon the partial pressure of gases in the blood and water afferent and efferent to the gills of rainbow trout. *Journal of Experimental Biology* **46**, 317-327

Huang, Q., Zhang, Y., Liu, S., Wang, W. and Luo, Y. (2013). Intraspecific scaling of the resting and maximum metabolic rates of the crucian carp (*Carassius auratus*). *PLoS ONE* **8**, e82837

Hudson, R.C.L. (1973). On the function of the white muscles in teleosts at intermediate swimming speeds. *Journal of Experimental Biology* **58**, 509-522

Hughes, G.M. (1973). Respiratory responses to hypoxia in fish. *American Zoologist* **13**, 475-489

Hughes, G.M. (1981). Effects of low oxygen and pollution on the respiratory systems of fish. In *Stress and Fish*. (ed. Pickering, A.D.). Academic Press, New York. pp.121-146

Hughes, G.M. and Saunders, R.L. (1970). Responses of the respiratory pumps to hypoxia in rainbow trout. *Journal of Experimental Biology* **53**, 529-545

Huss, H.H. (1995). Quality and Quality Changes in Fresh Fish. FAO Fisheries Technical Paper, No. 348. Food and Agriculture Organisation of the United Nations, Rome. 195p.

Iremonger, G.S. (2008). Smoltification and growth retardation in New Zealand King salmon *Oncorhynchus tshawyscha* (Walbaum). MSc thesis, University of Canterbury, Christchurch. 143p.

Ishibashi, Y., Inoue, K., Nakatsukasa, H., Ishitani, Y., Miyashita, S. and Murata, O. (2005). Ontogeny of tolerance to hypoxia and oxygen consumption of larval and juvenile red sea bream, *Pagrus major*. *Aquaculture* **244**, 331-340

Iversen, N.K., McKenzie, D.J., Malte, H. and Wang, T. (2010). Reflex bradycardia does not influence oxygen consumption during hypoxia in the European eel (*Anguilla anguilla*). *Journal of Comparative Physiology* **180**, 495-502

Jain, K.E. and Farrell, A.P. (2003). Influence of seasonal temperature on the repeat swimming performance of rainbow trout *Oncorhynchus mykiss*. *Journal of Experimental Biology* **206**, 3569-3579

Jain, K.E., Hamilton, J.C. and Farrell, A.P. (1997). Use of a ramp velocity test to measure critical swimming speed in rainbow trout (*Oncorhynchus mykiss*). *Comparative Biochemistry and Physiology* **117A**, 441-444

Jain, K.E., Birtwell, I.K. and Farrell, A.P. (1998). Repeat swimming performance of mature sockeye salmon following a brief recovery period: a proposed measure of fish health and water quality. *Canadian Journal of Zoology* **76**, 1488-1496

Janssen, G.J.A., Jerrett, A.R., Black, S.E. and Forster, M.E. (2010). The effects of progressive hypoxia and re-oxygenation on cardiac function, white muscle perfusion and haemoglobin saturation in anaesthetised snapper (*Pagrus auratus*). *Journal of Comparative Physiology B* **180**, 503-510

Jerrett, A.R., Holland, A.J. and Cleaver, S.E. (1998). Rigor contractions in "rested" and "partially exercised" Chinook salmon white muscle as affected by temperature. *Journal of Food Science* **63**, 53-56

Jobling, M. (1982). A study of some factors affecting rates of oxygen consumption of plaice, *Pleuronectes platessa* L. *Journal of Fish Biology* **20**, 501-516

Jobling, M. (1993). Bioenergetics: feed intake and energy partitioning. In *Fish Ecophysiology*. Fish and Fisheries Series, Vol. 9 (eds. Rankin, C.J. and Jensen, F.B.). Chapman and Hall, London. pp. 1-44

Jobling, M. (1994). *Fish Bioenergetics*. Fish and Fisheries Series, Vol. 13. Chapman and Hall, London. 309p.

Johnston, I.A. (1993). Phenotypic plasticity of fish muscle to temperature change. In *Fish Ecophysiology*. Fish and Fisheries Series, Vol. 9 (eds. Rankin, C.J. and Jensen, F.B.). Chapman and Hall, London. pp. 322-340

Johnston, I.A. and Dunn, J. (1997). Temperature acclimation and metabolism in ectotherms with particular reference to teleost fish. In *Temperature and Animal Cells.* (eds. Bowler, K. and Fuller, B.J.). Society of Experimental Biology, Cambridge. pp. 67-91

Johnston, I.A. and Goldspink, G. (1973). Quantitative studies of muscle glycogen utilisation during sustained swimming in crucian carp (*Carassius carassius* L.). *Journal of Experimental Biology* **59**, 607-615

Johnston, I.A. and Moon, T.W. (1980). Endurance exercise training in the fast and slow muscles of a teleost fish (*Pollachius virens*). *Journal of Comparative Physiology* **135**, 147-156

Johnston, I.A., Ward, P.S. and Goldspink, G. (1975). Studies on the swimming musculature of the rainbow trout. I. Fibre types. *Journal of Fish Biology* **7**, 451-458

Johnston, I.A., Davison, W. and Goldspink, G. (1977). Energy metabolism of carp swimming muscles. *Journal of Comparative Physiology* **114**, 203-216

Johnston, I.A., Clarke, A. and Ward, P. (1991). Temperature and metabolic rate in sedentary fish from the Antarctic, North Sea and Indo-West Pacific Ocean. *Marine Biology* **109**, 191-195

Jones, D.R. (1971). The effect of hypoxia and anaemia on the swimming performance of rainbow trout (*Salmo gairdneri*). *Journal of Experimental Biology* **55**, 541-551

Jones, D.R. (1982). Anaerobic exercise in teleost fish. *Canadian Journal of Zoology* **60**, 1131-1134

Jones, D.R. and Randall, D.J. (1978). The respiratory and circulatory systems during exercise. In *Fish Physiology. Vol. 7. Locomotion* (eds. Hoar, W.S. and Randall, D.J.). Academic Press, New York. pp. 425-501

Jones, D.R., Kiceniuk, J.W. and Bamford, O.S. (1974). Evaluation of the swimming performance of several fish species from the MacKenzie River. *Journal of the Fisheries Research Board of Canada* **31**, 1641-1647

Jourdan-Pineau, H., Dupont-Prinet, A., Claireaux, G. and McKenzie, D.J. (2010). An investigation of metabolic prioritisation in the European sea bass, *Dicentrarchus labrax. Physiological and Biochemical Zoology* **83**, 68-77

Kalinin, A.L., Rantin, F.T. and Glass, M.L. (1993). Dependence on body size of respiratory function in Hoplias malabaricus (Teleostei, Erythrinidae) during graded hypoxia. *Fish Physiology and Biochemistry* **12** 47-51

Kam, J.C. and Milligan, C.L. (2006). Fuel use during glycogenesis in rainbow trout (*Oncorhynchus mykiss* Walbum) white muscle studied *in vitro*. *Journal of Experimental Biology* **209**, 871-880

Kelleher, K. (2005). Discards in the World's Marine Fisheries: An Update. FAO Fisheries Technical Paper, No. 470. Food and Agriculture Organisation of the United Nations, Rome. 152p.

Kieffer, J.D. (2000). Limits to exhaustive exercise in fish. *Comparative Biochemistry and Physiology Part A* **126**, 161-179

Kieffer, J.D., Currie, S. and Tufts, B.L. (1994). Effects of environmental temperature on the metabolic and acid-base responses of rainbow trout to exercise. *Journal of Experimental Biology* **194**, 299-317

Kieffer, J.D., Ferguson, R.A., Tompa, J.E. and Tufts, B.L. (1996). Relationship between body size and anaerobic metabolism in brook trout and largemouth bass. *Transactions of the American Fisheries Society* **125**, 760-767

Kieffer, J.D., Alsop, D. and Wood, C.M. (1998). A respirometric analysis of fuel use during aerobic swimming at different temperatures in rainbow trout (*Oncorhynchus mykiss*). *Journal of Experimental Biology* **201**, 3123-3133

Kieffer, J.D., Kassie, R.S. and Taylor, S.G. (2011). The effects of low-speed swimming following exhaustive exercise on metabolic recovery and swimming performance in brook trout (*Salvelinus fontinalis*). *Physiological and Biochemical Zoology* **84**, 385-393

Kiessling, A., Espe, M., Ruohonen, K. and Mørkøre, T. (2004). Texture, gaping and colour of fresh and frozen Atlantic salmon flesh as affected by pre-slaughter iso-eugenol or CO_2 anaesthesia. *Aquaculture* **236**, 645-657

Killen, S.S., Costa, I., Brown, J.A. and Gamperl, A.K. (2007). Little left in the tank: metabolic scaling in marine teleosts and its implications for aerobic scope. *Proceedings of the Royal Society B* **274**, 431-438

Kita, J. and Itazawa, Y. (1989). Release of erythrocytes from the spleen during exercise and splenic constriction by adrenaline infusion in rainbow trout. *Japanese Journal of Icthyology* **36**, 48-52

Klieiber, M. (1932). Body size and metabolism. *Hilgardia* **6**, 316-353

Knox, D., Walton, M.J. and Cowey, C.B. (1980). Distribution of enzymes of glycolysis and gluconeogenesis in fish tissues. *Marine Biology* **56**, 7-10

Knudsen, P.K. and Jensen, F.B. (1998). Effects of exhausting exercise and catecholamines on K^+ balance, acid-base status and blood respiratory properties in carp. *Comparative Biochemistry and Physiology*, **119A**, 301-307.

Kolok, A.S. (1992). The swimming performances of individual largemouth bass (*Micropterus salmoides*) are repeatable. *Journal of Experimental Biology* **170**, 265-270

Kolok, A.S. (1999). Interindividual variation in the prolonged locomotor performance of ectothermic vertebrates: a comparison of fish and herpetofaunal methodologies and a brief review of the recent fish literature. *Canadian Journal of Fisheries and Aquatic Sciences* **56**, 700-710

Kolok, A.S. and Farrell, A.P. (1994). The relationship between maximum cardiac output and swimming performance in northern squawfish, *Ptychocheilus oregonensis*: the effect of coronary artery ligation. *Canadian Journal of Zoology* **72**, 1687-1690

Kutty, M.N. and Saunders, R.L. (1973). Swimming performance of young Atlantic salmon (*Salmo salar*) as affected by reduced ambient oxygen concentration. *Journal of the Fisheries Reach Board of Canda* **30**, 223-227

Laberee, K. and Milligan, C.L. (1999). Lactate transport across sarcolemmal vesicles isolate from rainbow trout white muscle. *Journal of Experimental Biology* **202**, 2167-2175

Larimore, R.W. and Duever, M.J. (1968). Effects of temperature acclimation on the swimming ability of smallmouth bass fry. *Transactions of the American Fisheries Society* **97**, 175-184

Lee, C.G., Devlin, R.H. and Farrell, A.P. (2003*a*). Swimming performance, oxygen consumption and excess post-exercise oxygen consumption in adult transgenic and ocean-ranched coho salmon. *Journal of Fish Biology* **62**, 753-766

Lee, C.G., Farrell, A.P., Lotto, A., Hinch, S.G., and Healy, M.C. (2003*b*). Excess post-exercise oxygen consumption in adult sockeye (*Oncorhynchus nerka*) and coho (*O. kisutch*) salmon following critical swimming speed. *Journal of Experimental Biology* **206**, 3253-3260

Lee, C.G., Farrell, A.P., Lotto, A., MacNutt, M.J., Hinch, S.G. and Healy, M.C. (2003*c*). The effect of temperature on swimming performance and oxygen consumption in adult sockeye (*Oncorhynchus nerka*) and coho (*O. kisutch*) salmon stocks. *Journal of Experimental Biology* **206**, 3239-3251

Lee-Jenkins, S.S.Y., Binder, T.R., Karch, A.P. and McDonald, D.G. (2007). The recovery of locomotory activity following exhaustive exercise in juvenile rainbow trout (*Oncorhynchus mykiss*). *Physiological and Biochemical Zoology* **80**, 88-98

Lefrançois, C. and Claireaux, G. (2003). Influence of ambient oxygenation and temperature on metabolic scope and scope for heart rate in the common sole *Solea solea*. *Marine Ecology Progress Series* **259**, 273-284

Legate, N.J., Bonen, A. and Moon, T.W. (2001). Glucose tolerance and peripheral glucose utilisation in rainbow trout (*Oncorhynchus mykiss*), American eel (*Anguilla rostrata*) and black bullhead catfish (*Ameiurus melas*). *General and Comparative Endocrinilogy* **122**, 48-59

Lewis, J.G., Manley, L., Whitlow, J.C. and Elder, P.A. (1992). Production of a monoclonal antibody to cortisol: application to a direct enzyme-linked immunosorbent assay of plasma. *Steroids* **57**, 82-85

Li, X.M., Cao, Z.D., Peng, J.L. and Fu, S.J. (2010). The effect of exercise training on the metabolic interaction between digestion and locomotion in juvenile darkbarbel catfish (*Peltebagrus vachelli*). *Comparative Biochemistry and Physiology* **156A**, 67-73

Lindsey, C.C. (1978). Form, Function and Locomotory Habits in Fish. In *Fish Physiology. Vol. 7. Locomotion.* (eds. Hoar, W.S. and Randall, D.J.). Academic Press, New York. pp. 1-100

Lockwood, S.J., Pawson, M.G. and Eaton, D.R. (1983). The effects of crowding in mackerel (*Scomber scomburs* L.) – physical condition and mortality. *Fisheries Research* **2**, 12-147

Lowe, T.E., Ryder, J.M., Carragher, J.F. and Wells, R.M.G. (1993). Flesh quality in snapper, *Pagrus auratus*, affected by capture stress. *Journal of Food Science* **58**, 770-773

MacNutt, M.J., Hinch, S.G., Farrell, A.P. and Topp, S. (2004). The effect of temperature and acclimation period on repeat swimming performance in cutthroat trout. *Journal of Fish Biology* **65**, 324-353

Magnuson, J.J. (1978). Locomotion in scombrid fishes: hydrodynamics, morphology and behaviour. In *Fish Physiology. Vol. 7. Locomotion* (eds. Hoar, W.S. and Randall, D.J.). Academic Press, New York. pp. 239-313

Mallekh, R. and Lagardère, J.P. (2002). Effect of temperature and dissolved oxygen concentration on the metabolic rate of the turbot and the relationship between metabolic rate and feeding. *Journal of Fish Biology* **60**, 1105-1115

Marras, S., Claireaux, G., McKenzie, D.J., Nelson, J.A. (2010). Individual variation and repeatability in aerobic and anaerobic swimming performance of European sea bass, *Dicentrarchus labrax*. *Journal of Experimental Biology* **213**, 26-32

Marvin, D.E. and Heath, A.G. (1968). Cardiac and respiratory responses to gradual hypoxia in three ecologically distinct species of freshwater fish. *Comparative Biochemistry and Physiology* **27**, 349-355

Mateus, C.S., Quintella, B.R. and Almeida, P.R. (2008). The critical swimming speed of Iverian barbell *Barbus bocagei* in relation to size and sex. *Journal of Fish Biology* **73**, 1783-1789

McFarlane, W.J. and McDonald, D.G. (2002). Relating intramuscular fuel use to endurance in juvenile rainbow trout. *Physiological and Biochemical Zoology* **75**, 250-259

McKenzie, D.J., Pedersen, P.B. and Jokumsen, A. (2007). Aspects of respiratory physiology and energetic in rainbow trout (*Oncorhynchus mykiss*) families with different size-at-age and condition factor. *Aquaculture* **263**, 280-294

McKenzie, D.J., Steffensen, J.F., Taylor, E.W. and Abe, A.S. (2012). The contribution of air breathing to aerobic scope and exercise performance in the banded knifefish *Gymnotus carapo* L. *Journal of Experimental Biology* **215**, 1323-1330

McKenzie, D.J., Wong, S., Randall, D.J., Egginton, S., Taylor, E.W. and Farrell, A.P. (2004). The effects of sustained exercise and hypoxia upon oxygen tensions in the red muscle of rainbow trout. *Journal of Experimental Biology* **207**, 3629-3637

Meka, J.M. and McCormick, S.D. (2005). Physiological response of wild rainbow trout to angling: impact of angling duration, fish size, body condition, and temperature. *Fisheries Research* **72**, 311-322

Meyer, W.F. and Cook, P.A. (1996). An assessment of the use of low-level aerobic swimming in promoting recovery from handling stress in rainbow trout. *Aquaculture International* **4**, 169-174

Meyler, L. (2008). Simulation of Net Structures Hydrodynamic Fields. In *Modelling and Simulation*. (eds. Petrone, G. and Cammarata, G.). Itech Education and Publishing, Rijeka. pp. 261-282

Milligan, C.L. (1996). Metabolic recovery from exhaustive exercise in rainbow trout. *Comparative Biochemistry and Physiology* **113A**, 51-60

Milligan, C.L. (2003). A regulatory role for cortisol in muscle glycogen metabolism in rainbow trout *Oncorhynchus mykiss* Walbaum. *Journal of Experimental Biology* **206**, 3167-3173

Milligan, C.L. and Girard, S.S. (1993). Lactate metabolism in rainbow trout. *Journal of Experimental Biology* **180**, 175-193

Milligan, C.L. and McDonald, D.G. (1988). *In vivo* lactate kinetics at rest and during recovery from exhaustive exercise in coho salmon (*Oncorhynchus kisutch*) and starry flounder (*Platichthys stellatus*). *Journal of Experimental Biology* **135**, 119-131

Milligan, C.L. and Wood, C.M. (1986). Tissue intracellular acid-base status and the fate of lactate after exhaustive exercise in the rainbow trout. *Journal of Experimental Biology* **123**, 123-144

Milligan, C.L. and Wood, C.M. (1987). Effects of strenuous activity on intracellular and extracellular acid-base status and H^+ exchange with the environment in the inactive, benthic starry flounder, *Platichthys stellatus*. *Physiological Zoology* **60**, 37-53

Milligan, C.L., Hooke, G.B. and Johnson, C. (2000). Sustained swimming at low velocity following a bout of exhaustive exercise enhances metabolic recovery in rainbow trout. *Journal of Experimental Biology* **203**, 921-926

Minhorst, A. and Liebezeit, G. (2003). Determination of glycogen in tissues of the blue mussel *Mytilus edulis* L. *Senckenbergiana maritime* **32**, 191-193

Ministry of Fisheries. (2008). The State of Our Fisheries 2008. Ministry of Fisheries, Wellington

Ministry of Fisheries. (2009). Snapper (SNA) Plenary Documents. Ministry of Fisheries, Wellington

Ministry of Fisheries. (2011). Ministry of Fisheries Annual Report, 2010/11. Ministry of Fisheries, Wellington

Ministry for Primary Industries. (2013*a*). Review of sustainability and other management controls for snapper 1 (SNA1). MPI Discussion Document No. 2013/31. Ministry for Primary Industries, Wellington

Ministry for Primary Industries. (2013*b*). SNAPPER 1. Ministry for Primary Industries, Wellington. http://www.fish.govt.nz/NR/rdonlyres/FA9FE90F-7AA8-4544-945A-B0EF4BE49343/0/2013Snapperfaqfactsheet_8.pdf

Ministry for Primary Industries. (2013*c*). The Status of New Zealand's Fisheries, 2013. Ministry for Primary Industries, Wellington

Ministry for Primary Industries. (2014). Snapper (SNA): Catch. Ministry for Primary Industries, Wellington. http://fs.fish.govt.nz/Page.aspx?pk=7&tk=100&sc=SNA

Mommsen, T.P., Vijayan, M.M. and Moon, T.W. (1999). Cortisol in teleosts: dynamics, mechanisms of action and metabolic regulation. *Reviews in Fish Biology and Fisheries* **9**, 211-268

Moon, T.W. (2001). Glucose intolerance in teleost fish: fact or fiction. *Comparative Biochemistry and Physiology Part B* **129**, 243-249

Morgan, J.D. and Iwama, G.K. (1997). Measurements of stressed states in the field. In *Fish Stress and Health in Aquaculture*. Society for Experimental Biology Seminar Series 62. (eds. Iwama, G.K., Pickering, A.D., Sumpter, J.P. and Schreck, C.B.). Cambridge University Press, Cambridge. pp. 247-268

Mossman, S. (2008). *Snapper: New Zealand's Greatest Fish*. AUT Media, Auckland, New Zealand. 189p.

Moyes, C.D. and West, T.G. (1995). Exercise metabolism of fish. In *Biochemistry and Molecular Biology of Fishes Vol. 4.* (ed. Hochachka, P.W. and Mommsen, T.P.). Elsevier Science, Amsterdam. pp 367-392

Mutungi, G. and Johnston, I.A. (1988). Influence of pH and temperature on force development and shortening velocity in skinned muscle fibres from fish. *Fish Physiology and Biochemistry* **5**, 257-262

Muusze, B., Marcon, J., van den Thillart, G. and Almeida-Val, V. (1998). Hypoxia tolerance of Amazon fish: respirometry and energy metabolism of the cichlid *Astronotus ocellatus*. *Comparative Biochemistry and Physiology Part A* **120**, 151-156

Myers, R.A., Hutchings, J.A. and Barrowman, N.J. (1997). Why do fish stocks collapse? The example of cod in Atlantic Canada. *Ecological Applications* **7**, 91-106

Neilson, J.D., Waiwood, K.G. and Smith, S.J. (1989). Survival of Atlantic halibut (*Hippoglossus hippoglossus*) caught by longline and otter trawl gear. *Canadian Journal of Fisheries and Aquatic Sciences* **46**, 887-897

Nelson, J.A. (1989). Critical swimming speeds of yellow perch *Perca flavenscens*: comparison of populations from a naturally acidic lake and a circumneutral lake in acid and neutral water. *Journal of Experimental Biology* **145**, 239-254

Nelson, J.A., Tang, Y. and Boutilier, R.G. (1994). Differences in exercise physiology between two Atlantic cod (*Gadus morhua*) populations from different environments. *Physiological Zoology* **67**, 330-354

Nelson, J.A., Gotwalt, P.S., Reidy, S.P. and Webber, D.M. (2002). Beyond U_{crit}: matching swimming performance tests to the physiological ecology of the animal, including a new fish "drag strip." *Comparative Biochemistry and Physiology* **133**, 289-302

Neumann, P., Holeton, G.F. and Heisler, N. (1983). Cardiac output and regional blood flow in gills and muscles after exhaustive exercise in rainbow trout (*Salmo gairdneri*). *Journal of Experimental Biology* **105**, 1-14

Nikinmaa, M. and Huestis, W.H. (1984). Adrenergic swelling of nucleated erythrocytes: cellular mechanisms in a bird, domestic goose, and two teleosts, striped bass and rainbow trout. *Journal of Experimental Biology* **113**, 215-224

Nilsson, S. and Grove, D.J. (1974). Adrenergic and cholinergic innervations of the spleen of the cod (*Gadus morhua*). *European Journal of Pharmacology* **28**, 135-143

Nilsson, G.E. and Östlund-Nilsson, S. (2008). Does size matter for hypoxia tolerance in fish? *Biology Reviews* **83**, 173-189

Norin, T. and Malte, H. (2012). Intraspecific variation in aerobic metabolic rate of fish: relations with organ size and enzyme activity in brow trout. *Physiological and Biochemical Zoology* **85**, 645-656

O' Connor, K.I., Taylor, A.C. and Metcalfe, N.B. (2000). The stability of standard metabolic rate during a period of food deprivation in juvenile Atlantic salmon. *Journal of Fish Biology* **57**, 41-51

Oddsson, G., Pikitch, E.K., Dickhoff, W. and Erickson, D.L. (1994). Effects of towing, sorting and caging on physiological stress indicators and survival in trawl caught and discarded Pacific halibut (*Hippoglossus stenolepis* Schmidt 1904). In *High Performance Fish: Proceedings of the Fish Physiology Symposium, Vancouver, Canada, 16-21 July 1994*. (ed. MacKinley, D.D.). Fish Physiology Association, Vancouver. pp. 437-442

Oehlenschlager, J. and Rehbein, H. (2009). Basic facts and figures. In *Fishery Products: Quality, Safety and Authenticity* (eds. Rehbein, H and Oehlenschlager, J.). Wiley-Blackwell, Oxford. pp. 1-18

Oikawa, S., Takemori, M. and Itazawa, Y. (1992). Relative growth of organs and parts of a marine teleost, the porgy, *Pagrus major*, with special reference to metabolism-size relationships. *Japanese Journal of Icthyology* **39**, 243-249

Olla, B.L., Davis, M.W. and Schreck, C.B. (1992). Comparison of predator avoidance capabilities with corticosteroid levels induced by stress in juvenile coho salmon. *Transactions of the American Fisheries Society* **121**, 544-547

Olla, B.L., Davis, M.W. and Schrek, C.B. (1997). Effects of simulated trawling on sablefish and walleye pollock: the role of light intensity, net velocity and towing duration. *Journal of Fish Biology* **50,** 1181-1194

Olla, B.L., Davis, M.W. and Schreck, C.B. (1998). Temperature magnified post-capture mortality in adult sablefish after simulated trawling. *Journal of Fish Biology* **53**, 743-751

Olsen, R.E., Oppedal, F., Tenningen, M. and Vold, A. (2012). Physiological response and mortality caused by scale loss in Atlantic herring. *Fisheries Research* **129-130**, 21-27

Olsen, S.H., Tobiassen, T., Akse, L., Evensen, T.H. and Midling, K.O. (2013). Capture induced stress and live storage of Atlantic cod (*Gadus morhua*) caught by trawl: consequences for flesh quality. *Fisheries Research* **147**, 446-453

Ott, M.E., Heisler, N. And Ultsch, G.R. (1980). A re-evaluation of the relationship between temperature and the critical oxygen tension in freshwater fishes. *Comparative Physiology and Biochemistry* **67A**, 337-340

Oufiero, C.E and Garland, T. (2009). Repeatability and correlation of swimming performances and size over varying time-scales in the guppy (*Poecilia reticulate*). *Functional Ecology* **23**, 969-978

Pagnotta, A. and Milligan, C.L. (1991). The role of blood glucose in the restoration of muscle glycogen during recovery from exhaustive exercise in rainbow trout (*Oncorhynchus mykiss*) and winter flounder (*Pseudopleuronectes ameicanus*). *Journal of Experimental Biology* **161**, 489-508

Pagnotta, A., Brooks, L. and Milligan, C.L. (1994). The potential regulatory roles of cortisol in recovery from exhaustive exercise in rainbow trout. *Canadian Journal of Zoology* **72**, 2136-2146

Pain, R.H. (1987). Temperature and macromolecular structure and function. In *Temperature and Animal Cells.* (eds. Bowler, K. and Fuller, B.J.). Society of Experimental Biology, Cambridge. pp. 21-33.

Pankhurst, N.W. and Sharples, D.F. (1992). Effects of capture and confinement on plasma cortisol concentrations in the snapper, *Pagrus auratus*. *Australian Journal of Marine and Freshwater Research* **43**, 345-356

Parker, R.R. and Black, E.C. (1959). Muscular fatigue and mortality in troll-caught Chinook salmon (*Oncorhynchus tshawytscha*). *Journal of the Fisheries Research Board of Canada* **16**, 95-106

Parker, R.R., Black, E.C. and Larkin, P.A. (1959). Fatigue and mortality in troll-caught Pacific salmon (*Oncorhynchus*). *Journal of Fisheries Research Board of Canada* **16**, 429-448

Parker, S.J., Rankin, P.S., Hannah, R.W. and Schreck, C.B. (2003). Discard mortality of trawl-caught lingcod in relation to two duration and time on deck. *North American Journal of Fisheries Management* **23**, 530-542

Patel, B.N. (2011). The response of juvenile snapper, *Pagrus auratus*, to environmental hypoxia and oxygen profiles in the Mahurangi Estuary, Auckland region. MSc thesis, University of Auckland, Auckland.

Patterson, S., Johnston, I.A. and Goldspink, G. (1975). A histochemical study of the lateral muscles of five teleost species. *Journal of Fish Biology* **7**, 159-166

Paxton, R., Gist, D.H. and Umminger, B.L. (1984). Serum cortisol levels in thermally-acclimated goldfish (*Carassius auratus*) and killifish (*Fundulus heteroclitus*): implications in control of hepatic glycogen metabolism. *Comparative Biochemistry and Physiology* **78B**, 813-816

Peacey, J. (2003). Managing Catch Limits in Multispecies ITQ Fisheries, New Zealand. Ministry of Fisheries, Wellington.

Peake, S.J. (2008). Swimming performance and behaviour of fish species endemic to Newfoundland and Labrador: A literature review for the purpose of establishing design and water velocity criteria for fishways and culverts. Canadian Manuscript Reports of Fisheries and Aquatic Sciences, Vol. 2843. 52p.

Peake, S.J. and Farrell, A.P. (2006). Fatigue is a behavioural response in respirometer-confined smallmouth bass. *Journal of Fish Biology* **68**, 1742-1755

Pearson, M.P. and Stevens, E.D. (1991). Size and haematological impact of the splenic erythrocyte reservoir in rainbow trout, *Oncorhynchus mykiss*. *Fish Physiology and Biochemistry* **9**, 39-50

Pearson, M.P., van der Kraak, G. and Stevens, E.D. (1992). *In vivo* pharmacology of spleen contraction in rainbow trout. *Canadian Journal of Zoology* **70**, 625-627

Peck, L.S., Webb, K.E. and Bailey, D.M. (2004). Extreme sensitivity of biological function in Antarctic marine species. *Functional Ecology* **18**, 625-630

Perry, S.F., Jonz, M.G. and Gilmour, K.M. (2009). Oxygen sensing and the hypoxic ventilatory response. In *Fish Physiology, Vol. 27. Hypoxia.*. (eds. Richards, J.G., Farrell, A.P. and Brauner, C.J.). Elsevier, London. pp. 193-253.

Petersen, R.H. (1974). Influence of fenitrothion on swimming velocity of brook trout (*Salvelinus fontinalis*). *Journal of the Fisheries Research Board of Canada* **31**, 1757-1762

Petersen, L.H. and Gamperl, A.K. (2010). Effect of acute and chronic hypoxia on the swimming performance, metabolic capacity and cardiac function of Atlantic cod (*Gadus morhua*). *Journal of Experimental Biology* **213**, 808-819

Pickering, A.D., Pottinger, T.G. and Christie, P. (1982). Recovery of the brown trout *Salmo trutta* L. from acute handling stress: a time-course study. *Journal of Fish Biology* **20**, 229-244

Plante S., Chabot, D. and Dutil, J.D. (1998). Hypoxia tolerance in Atlantic cod. *Journal of Fish Biology* **53**, 1342-1356

Plaut, I. (2001). Critical swimming speed: its ecological relevance. *Comparative Biochemistry and Physiology Part A* **131**, 41-50

Pörtner, H.O. and Grieshaber, M.K. (1993). Critical PO_2(s) in oxyconforming and oxyregulating animals: gas exchange, metabolic rate and the mode of energy production. In *The Vertebrate Gas Transport Cascade: Adaptations to Environment and Mode of Life*. (eds. Hochachka, P.W., Lutz, P.L., Rosenthal, M. and van den Thillardt, G.). CRC Press, Boca Raton. pp. 330-357

Post, J.R. and Lee, J.A. (1996). Metabolic ontogeny of teleost fishes. *Canadian Journal of Fisheries and Aquatic Science* **53**, 910-923

Powell, M.D. and Nowak, B.F. (2003). Acid-base and respiratory effects of confinement in Atlantic salmon affected with amoebic gill disease. *Journal of Fish Biology* **62**, 51-63

Primmett, D.R.N., Randall, D.J., Mazeaud, M. and Boutilier, R.G. (1986). The role of catecholamines in erythrocyte pH regulation and oxygen transport in rainbow trout (*Salmo gairdneri*) during exercise. *Journal of Experimental Biology* **122**, 139-148

Pritchard, A.W., Hunter, J.R. and Lasker, R. (1971). The relation between exercise and biochemical changes in red and white muscle and liver in the jack mackerel, *Trachurus symmetricus*. *Fisheries Bulletin* **69**, 379-386

Randall, D.J. (1982). The control of respiration and circulation in fish during exercise and hypoxia. *Journal of Experimental Biology* **100**, 275-288

Randall, D.J. and Brauner, C. (1991). Effects of environmental factors on exercise in fish. *Journal of Experimental Biology* **160**, 113-126

Randall, D.J. and Daxboeck, C. (1982). Cardiovascular changes in the rainbow trout (*Salmo gairdneri* Richardson) during exercise. *Canadian Journal of Zoology* **60**, 1135-1140

Randall, D.J. and Smith, J.C. (1967). The regulation of cardiac activity in a hypoxic environment. *Physiological Zoology* **40**, 104-113

Randall, D.J., Mense, D. and Boutilier, R.G. (1987). The effects of burst swimming on aerobic swimming in Chinook salmon (*Oncorhynchus tshawytscha*). *Marine Behaviour and Physiology* **13**, 77-88

Rantin, F.T., Kalinin, A.L., Glass, M.L. and Fernandes M.N. (1992). Respiratory responses to hypoxia in relation to mode of life of two erythrinid species (*Hoplias malabaricus* and *Hoplias lacerdae*). *Journal of Fish Biology* **41**, 805-912

Rao, G.M.M. (1968). Oxygen consumption of rainbow trout (*Salmo gairdneri*) in relation to activity and salinity. *Canadian Journal of Zoology* **46**, 781-786

Rao, G.M.M. (1971). Influence of activity and salibity on the weight-dependent oxygen consumption of the rainbow trout *Salmo gairdneri*. *Marine Biology* **8**, 205-212

Reidy, S.P., Nelson, J.A., Tang, Y. and Kerr, S.R. (1995). Post-exercise metabolic rate in Atlantic cod and its dependence upon method of exhaustion. *Journal of Fish Biology* **47**, 377-386

Rice, J.A. (1990). Bioenergetics modelling approaches to evaluation of stress in fishes. *American Fisheries Society Symposium* **8**, 80-92

Richards, J.G. (2009) Metabolic and molecular response of fish to hypoxia In *Fish Physiology, Vol. 27. Hypoxia*.. (eds. Richards, J.G., Farrell, A.P. and C.J. Brauner). Elsevier, London. pp. 443-485.

Richards, J.G., Heigenhauser, G.J.F. and Wood, C.M. (2002*a*). Lipid oxidation fuels recovery from exhaustive exercise in white muscle of rainbow trout. *American Journal of Physiology:* **282**, R89-R99

Richards, J.G., Mercado, A.J., Clayton, C.A., Heigenhauser, G.J.F. and Wood, C.M. (2002*b*). Substrate utilisation during graded aerobic exercise in rainbow trout. *Journal of Experimental Biology* **205**, 2067-2077

Robb, T. and Abrahams, M.V. (2003). Variation in tolerance to hypoxia in a predator and prey species: an ecological advantage of being small? *Journal of Fish Biology* **62**, 1067-1081

Robb, D.H.F., Kestin, S.C. and Warriss, P.D. (2000). Muscle activity at slaughter. I. Changes in flesh colour and gaping in rainbow trout. *Aquaculture* **182**, 261-269

Robinson, E.E. (2008). Antarctic Fish: Thermal Specialists or Adaptable Generalists? PhD thesis, University of Canterbury, Christchurch. 224p.

Rome, L.C., Loughna, P.T. and Goldspink, G. (1984). Muscle fibre activity in carp as a function of swimming speed and muscle temperature. *American Journal of Physiology* **247**, R272-R279

Rome, L.C., Loughna, P.T. and Goldspink, G. (1985). Temperature acclimation improved sustained swimming performance in carp at low tempearutres. *Science* **228**, 194-196

Rome, L.C., Funke, R.P. and Alexander, R.M. (1990). The influence of temperature on muscle velocity and sustained performance in swimming carp. *Journal of Experimental Biology* **154**, 163-178

Rome, L.C., Choi, I.N., Lutz, G. and Sosnicki, A. (1992). The influence of temperature on muscle function in the fast swimming scup. I. Swimming velocity and muscle recruitment during swimming. *Journal of Experimental Biology* **163**, 259-279

Rome, L.C., Swank, D., and Corda, D. (1993). How fish power swimming. *Science* **261**, 340-343

Rotabakk, B.T., Skipnes, D., Akse, L. and Birkeland, S. (2011). Quality assessment of Atlantic cod (*Gadus morhua*) caught by longlining and trawling at the same time and location. *Fisheries Research* **112**, 44-51

Roth, B., Slinde, E. and Arildsen, J. (2006). Pre or post mortem muscle activity in Atlantic salmon (*Salmo salar*). The effects of rigor mortis and the physical properties of flesh. *Aquaculture* **257**, 504-510

Rubner, M. (1883). Ueber den einfluss der Körpergrösse auf Stoff-und Kraftwechsel. *Zeitschriftfür Biologie* **19**, 535-62

Ryer, C.H. (2002). Trawl stress and escapee vulnerability to predation in juvenile walleye pollock: is there an unobserved bycatch of behaviourally impaired escapees? *Marine Ecology Progress Series* **232**, 269-279

Ryer, C.H. (2004). Laboratory evidence for behavioural impairment of fish escaping trawls: a review. *ICES Journal of Marine Science* **61**, 1157-1164

Ryer, C.H., Ottmar, M.L. and Sturm, E.A. (2004). Behavioural impairment after escape from trawl codends may not be limited to fragile species. *Fisheries Research* **66**, 261-269

Saito, T., Arai, K. and Yajima, T. (1959). Changes in purine nucleotides of red lateral muscle of rainbow trout. *Nature* **184**, 1415

Sänger, A.M. (1992). Quantitative fine structural diversification of red and white muscle fibres in cyprinids. *Environmental Biology of Fishes* **33**, 97-104

Sangster, G.I., Lehmann, K. and Breen, M. (1996). Commercial fishing experiments to assess the survival of haddock and whiting after escape from four sizes of diamond mesh cod-ends. *Fisheries Research* **25**, 323-345

Scarabello, M., Wood, C.M. and Heigenhauser, G.J.F. (1991). Glycogen depletion in juvenile rainbow trout as an experimental test of the oxygen debt hypothesis. *Canadian Journal of Zoology* **69**, 2562-2568

Scarabello, M., Heigenhauser, G.J.F. and Wood, C.M. (1992). Gas exchange, metabolite status and excess post-exercise oxygen consumption after repetitive bouts of exhaustive exercise in rainbow trout. *Journal of Experimental Biology* **167**, 155-169

Schreer, J.F., Resch, D.M., Gately, M.L. and Cooke, S.J. (2005). Swimming performance of brook trout after simulated catch-and-release angling: looking for air exposure thresholds. *North American Journal of Fisheries Management* **25**, 1513-1517

Schmidt-Nielsen, K. (1977). Problems of Scaling: Locomotion and Physiological Correlates. In *Scale Effects in Animal Locomotion.* (ed. Pedley, P.J.). Academic Press, London. pp. 1-21

Schmidt-Nielsen, K. (1997). *Animal Physiology*. 5th edition. Cambridge University Press, Cambridge. 607p.

Schulte, P.M., Moyes, C.M. and Hochachka, P.W. (1992). Integrating metabolic pathways in post-exercise recovery of white muscle. *Journal of Experimental Biology* **166**, 181-192

Schulte, P.M., Healy, T.M. and Fangue, N.A. (2011). Thermal performance curves, phenotypic plasticity and the time scales of temperature exposure. *Integrative and Comparative Biology* **51**, 691-702

Schurmann, H. and Steffensen, J.F. (1994). Spontaneous swimming activity of Atlantic cod *Gadus morhua* exposed to graded hypoxia at three temperatures. *Journal of Experimental Biology* **197**, 129-142

Schurmann, H. and Steffensen, J.F. (1997). Effects of temperature, hypoxia and activity on the metabolism of juvenile Atlantic cod. *Journal of Fish Biology* **50,** 1166-1180

Seifter, S., Dayton, B.S., Novic, B. and Muntwyler, E. (1950). The estimation of glycogen with the anthrone reagent. *Archives of Biochemistry* **25**, 191-200

Sepulveda, C. and Dickson, K.A. (2000). Maximum sustainable swimming speeds and cost of swimming in kawakawa tuna (*Euthynnus affinis*) and chub mackerel (*Scomber japonicas*). *Journal of Experimental Biology* **203**, 3089-3101

Sfakianakis, D.G. and Kentouri, M. (2010). Effect of temperature on muscle lactate metabolic recovery in sea bass (*Dicentrarchus labrax* L.) juveniles exposed to exhaustive exercise. *Fish Physiology and Biochemistry* **36**, 387-390

Sharpe, R. and Milligan, C.L. (2003). Lactate efflux from sarcolemmal vesicles isolated form rainbow trout *Oncorhynchus mykiss* white muscle is via simple diffusion. *Journal of Experimental Biology* **206**, 543-549

Sherwood, L., Klandorf, H. and Yancey, P.H. (2005). *Animal Physiology: from genes to organisms*. Thomson Brooks/Cole, Belmont.

Shimoda, L.A. and Polak, J. (2011). Hypoxia. 4. Hypoxia and ion channel function. *American Journal of Physiology* **300**, C951-C967

Sidell, B.D. and Moerland, T.S. (1989). Effects of temperature on muscular function and locomotory performance in teleost fish. In *Advances in Comparative and Environmental Physiology, Vol. 5.* (eds. Gilles, R., Butler, P.J., Greger, R., Mangum, C.P., Somero, G.C., Takahashi, K. and Weber, R.E.). Springer-Verlag, Berlin. pp.115-156

Sigholt, T., Erikson, U., Rustad, T., Johansen, T.S., Nordtvedt, T.S. and Seland, A. (1997). Handling stress and storage temperature affect meat quality of farmed-raised Atlantic salmon (*Salmo salar*). *Journal of Food Science* **62**, 898-905

Simonot, D.L. (2006). Cardiac remodelling in diploid and triploid rainbow trout (*Oncorhynchus mykiss* Walbaum). MSc thesis, University of British Colombia, Vancouver. 133p.

Sisson, J.E. and Sidell, B.D. (1987). Effect of thermal acclimation on muscle fibre recruitment of swimming striped bass (*Morone saxatilis*). *Physiological Zoology* **60**, 310-320

Sjøgaard, G. (1990). Role of exercise-induced potassium fluxes underlying muscle fatigue a brief review. *Canadian Journal of Physiology and Pharmacology* **69**, 238-245

Skjervold, P.O., Fjæra, S.O., Østby, P.B. and Einen, O. (2001). Live-chilling and crowding stress before slaughter of Atlantic salmon (*Salmo salar*). *Aquaculture* **192**, 265-280

Sloman, K.A., Wood, C.M., Scott, G.R., Wood, S., Kajimura, M., Johannsson, O.E., Almeida-Val, V.M.F. and Val, A.L. (2006). Tribute to R.G. Boutilier: The effect of size on the physiological and behavioural responses of Oscar, *Astronotus ocellatus*, to hypoxia. *Journal of Experimental Biology* **209**, 1197-1205

Statistics New Zealand. (2009). Fish Monetary Stock Amount: 1996-2008. Statistics New Zealand, Wellington.

Steffensen, J.F. (1989). Some errors in respirometry of aquatic breathers: how to avoid and correct for them. *Fish Physiology and Biochemistry* **6**, 49-59

Steffensen, J.F. and Farrell, A.P. (1998). Swimming performance, venous oxygen tension and cardiac performance of coronary-ligated rainbow trout, *Oncorhynchus mykiss*, exposed to progressive hypoxia. *Comparative Biochemistry and Physiology* **119A**, 585-592

Steinhausen, M.F., Sandblom, E., Eliason, E.J., Verhille, C. and Farrell, A.P. (2008). The effect of acute temperature increases in the cardiorespiratory performance of resting and swimming sockeye salmon (*Oncorhynchus nerka*). *Journal of Experimental Biology* **211**, 3915-3926

Stevens, E.D. and Black, E.C. (1966). The effect of intermittent exercise on carbohydrate metabolism in rainbow trout, *Salmo gairdneri*. *Journal of the Fisheries Research Board of Canada* **23**, 471-485

Stevens, E.D. and Randall, D.J. (1967). Changes in blood pressure, heart rate and breathing rate during moderate swimming activity in rainbow trout. *Journal of Experimental Biology* **46**, 307-315

Stewart, J. (2008). Capture depth related mortality of discarded snapper (*Pagrus auratus*) and implications for management. *Fisheries Research* **90**, 289-295

Storey, K.B. (1991). Metabolic consequences of exercise in organs of rainbow trout. *Journal of Experimental Zoology* **260**, 157-164

Strange, R.J. and Cech, J.J. (1992). Reduced swimming performance of striped bass after confinement stress. *Transactions of the American Fisheries Society* **121**, 206-210

Sumpton, W. and Jackson, S. (2005). The effects of incidental trawl capture of juvenile snapper *(Pagrus auratus*) on yield of a sub-tropical line fishery in Australia: an assessment examining habitat preference and early life history characteristics. *Fisheries Research* **71**, 335-347

Suski, C.D., Cooke, S.J. and Tufts, B.L. (2007). Failure of low-velocity swimming to enhance recovery from exhaustive exercise in largemouth bass (*Micropterus salmoides*). *Physiological and Biochemical Zoology* **80**, 78-87

Suuronen, P. (2005). Mortality of Fish Escaping Fishing Gears. FAO Fisheries Technical Paper, No. 478. Food and Agriculture Organisation of the United Nations, Rome. 86p.

Suuronen, P., Turunen, T., Kiviniemi, M. and Karjalainen, J. (1995). Survival of vendace (*Coregonus albula*) escaping from a trawl cod end. *Canadian Journal of Fisheries and Aquatic Sciences* **52**, 2527-2533

Suuronen, P., Erickson, D.L. and Orrensalo, A. (1996*a*). Mortality of herring escaping from pelagic trawl codends. *Fisheries Research* **25**, 305-321

Suuronen, P., Perez-Comas, J.A., Lehtonen, E. and Tschernij, V. (1996*b*). Size-related mortality of herring (*Clupea harengus* L.) escaping through a rigid sorting grid and trawl codend meshes. *ICES Journal of Marine Science* **53**, 691-700

Svendsen, J.C., Steffensen, J.F., Aarestrup, K., Frisk, M., Etzerodt, A. and Jyde, M. (2012). Excess post-hypoxic oxygen consumption in rainbow trout (*Oncorhynchus mykiss*): recovery in normoxia and hypoxia. *Canadian Journal of Zoology* **90**, 1-11

Taylor, S.E., Egginton, S. and Taylor, E.W. (1996). Seasonal temperature acclimation of rainbow trout: cardiovascular and morphometric influences on maximal sustainable exercise level. *Journal of Experimental Biology* **199**, 835-845

Taylor, E.W., Jordan, D. and Coote, J.H. (1999). Central control of the cardiovascular and respiratory systems and their interactions in vertebrates. *Physiological Reviews* **79**, 855-916

Taylor, E.W., Egginton, S., Taylor, S.E. and Butler, P.J. (2008). Factors which may limit swimming performance at different temperatures. In *Global Warming: Implications for Freshwater and Marine Fish.* Society for Experimental Biology Seminar Series 61. (eds. Wood, C.M. and McDonald, D.G.). Cambridge University Press, Cambridge. pp 105-133

Tejada, M. (2009). ATP-derived products and K-value determination. In *Fishery Products: Quality, Safety and Authenticity* (eds. Rehbein, H. and Oehlenschlager, J.). Wiley-Blackwell, Oxford. pp. 68-88

Thiele, W., Kroeger, M., Gau, S., Hopp., M. and Kluber, U. (1997). Flow measurements in a cod-end. International Council for the Exploration of the Sea, Fisheries Technology, CM 1997/FF:10. pp. 1-9

Thomas, P.M., Pankhurst, N.W. and Bremner, H.A. (1999). The effect of stress and exercise on post-mortem biochemistry of Atlantic salmon and rainbow trout. *Journal of Fish Biology* **54**, 1177-1196

Thorarensen, H. and Farrell, A.P. (2006). Postprandial intestinal blood flow, metabolic rates and exercise in Chinook salmon (*Oncorhynchus tshawytscha*). *Physiological and Biochemical Zoology* **79**, 688-694

Tuckey, N.P.L. (2008). Technologies for tissue preservation: the role of endogenous and exogenous antioxidants in preserving tissue function in Chinook salmon, *Oncorhynchus tshawytscha*. PhD thesis, University of Canterbury, Christchurch.

Tuckey, N.P.L., Forster, M.E. and Gieseg, S.P. (2010). Effects of rested harvesting on muscle metabolite concentrations and K-values in Chinook salmon (*Oncorhynchus tshawytscha*) fillets during storage at 15 ^{o}C. *Journal of Food Science* **75**, 459-464

Tuckey, N.P.L., Forgan, L.G. and Jerrett, A.R. (2012). Fillet colour correlates with biochemical status in Australian snapper (*Pagrus auratus*) during storage in refrigerated seawater. *Aquaculture* **356-357**, 256-263

Turner, J.D. and Wood, C.M. (1983). Factors affecting lactate and proton efflux from pre-exercised, isolated-perfused rainbow trout trunks. *Journal of Experimental Biology* **105**, 395-401.

Turunen, T., Kakela, A., and Hyvarinen, H. (1994). Trawling stress and mortality in undersized (<40cm) brown trout (*Salmo trutta* L.). *Fisheries Research* **19**, 51-64

van der Boon, J., van den Thillart, G.E.E. and Addink, A.D.F. (1991). The effects of cortisol administration on intermediary metabolism in teleost fish. *Comparative Biochemistry and Physiology* **100A**, 47-53

van Ginneken, V.J.T., van den Thillart, G.E.E., Addink, A.D.F. and Erkelens, C. (1995). Fish muscle energy metabolism measured during hypoxia and recovery: an *in vivo* ^{31}P-NMR study. *American Journal of Physiology* **268**, R1178-1187

van Ginneken, V.J.T., Addink A.D.F., van den Thilliart, G.R.R.J.M., Körner, F., Noldus, L. and Buma, M. (1997). Metabolic rate and level of activity determined in tilapia (*Oreochromis mossambicus* Peters) by direct and indirect calorimetry and videomonitoring. *Thermochimica Acta* **291**, 1-13

van Ginneken, G., Coldenhoff, K., Boot, R., Hollander, J., Lefeber, F. and van den Thillart, G. (2008). Depletion of high energy phosphates implicates post-exercise mortality in carp and trout; an *in vivo* ^{31}P-NMR study. *Comparative Biochemistry and Physiology Part A* **149**, 98-108

van Waversveld, J., Addink, A.D.F. and van den Thillart, G. (1989). Simultaneous direct and indirect calorimetry on normoxic and anoxic goldfish. *Journal of Experimental Biology* **142**, 325-335

Vandenboom, R. (2004). The myofibrillar complex and fatigue: a review. *Canadian Journal of Applied Physiology* **29**, 330-356

Vanquer-Sunyer, R. and Duarte, C.M. (2008). Thresholds of hypoxia for marine biodiversity. *Proceedings of the National Academy of Sciences* **105**, 15452-15457

Veiseth, E., Fjæra, S.O., Bjerkeng, B. and Skjervold, P.O. (2006). Accelerated recovery of Atlantic salmon (*Salmo salar*) from effects of crowding by swimming. *Comparative Biochemistry and Physiology Part B* **144**, 351-358

Verheyen, R., Blust, R. and Decleir, W. (1994). Metabolic rate, hypoxia tolerance and aquatic surface respiration of some lacustrine and riverine African cichlid fishes. *Comparative Biochemistry and Physiology* **107A** 403-411

Videler, J.J. (1993). *Fish swimming*. Fish and Fisheries Series, Vol. 10. Chapman and Hall, London. 260p.

Vijayan, M.M. and Moon, T.W. (1992). Acute handling stress alters hepatic glycogen metabolism in food deprived rainbow trout (*Oncorhynhus mykiss*). *Canadian Journal of Fisheries and Aquatic Sciences* **49**, 2260-2266

Vijayan, M.M., Ballantyne, J.S. and Leatherland, J.H. (1990). High stocking densities alters the energy metabolism of brook charr, *Salvelinus fontinalis. Aquaculture* **88**, 371-381

Vijayan, M.M., Ballantyne, J.S. and Leatherland, J.F. (1991). Cortisol-induced changes in some aspects of intermediary metabolism of *Salvelinus fontinalis*. *General and Comparative Endocrinology* **82**, 476-486

Vornanen, M., Stecyk, J.W. and Nilsson, G.E. (2009) The anoxia-tolerant crucian carp (*Carassius carassius* L.). In *Fish Physiology, Vol. 27. Hypoxia*.. (eds. Richards, J.G., Farrell, A.P. and Brauner, C.J.). Elsevier, London. pp. 397-441

Walrond, C. (2013). Coastal fish - New Zealand's coastal fish. *Te Ara - the Encyclopaedia of New Zealand*. www.TeAra.govt.nz/en/interactive/8810/sea-surface-temperatures.

Wang, Y., Heigenhausen, G.J.F., and Wood, C.M. (1994*a*). Integrated responses to exhaustive exercise and recovery in rainbow trout white muscle: acid-base, phosphogens, carbohydrate, lipid, ammonia, fluid volume and electrolyte metabolism. *Journal of Experimental Biology* **195**, 227-258

Wang, Y., Wilkie, M.P., Heigenhausen, G.J.F. and Wood, C.M. (1994*b*). The analysis of metabolites in rainbow trout white muscle: a comparison of different sampling and processing methods. *Journal of Fish Biology* **45**, 855-873

Wang, Y., Wright, P.M., Heigenhauser, G.J.F. and Wood, C.M. (1997). Lactate transport by rainbow trout white muscle: kinetic characteristics and sensitivity to inhibitors. *American Journal of Physiology* **272**, R1577-1587

Wang, T., Lefevre, S., Huong, D.T.T., Cong, N.V. and Bayley, M. (2009). The effects of hypoxia on growth and digestion. In *Fish Physiology, Vol. 27. Hypoxia*. (eds. Richards, J.G., Farrell, A.P. and Brauner, C.J.). Elsevier, London. pp. 361-396

Wardle, C.S. (1975). Limits of fish swimming speed. *Nature* **255**, 725-727

Wardle, C.S. (1978). Non-release of lactic acid from anaerobic swimming muscle of plaice *Pleuronectes platessa* L: a stress reaction. *Journal of Experimental Biology* **77**, 141-155

Wardle, C.S. (1993). Fish behaviour and fishing gear. In *Behaviour of Teleost Fishes.* Fish and Fisheries Series Vol. 7. 2nd edition. (ed. Pitcher, T.J.). Chapman and Hall, London. pp.609-643

Webb, P.W. (1977). Effects of size on performance and energetic of fish. In *Scale Effects in Animal Locomotion.* (ed. Pedley, T.J.). pp. 315-331. Academic Press, London, U.K.

Wedermeyer, G.A., Barton, B.A. and McLeay, D.J. (1990). Stress and acclimation. In *Methods for Fish Biology*. (eds. Schreck, C.B. and Moyle, P.B.) American Fisheries Society, Bethesda. pp. 451-489.

Weibel, E.R., Bacigalupe, L.D., Schmitt, B. and Hoppeler, H. (2004). Allometric scaling of maximal metabolic rate in mammals: muscle anaerobic capacity as determinant factor. *Respiratory Physiology* **140**, 115-132

Wells, R.M.G. (1987). Stress responses imposed by fish capture and handling: a physiological perspective. *Food Technology in Australia* **39**, 479-481

Wells, R.M.G. and Dunphy, B.J. (2009). Potential impact of metabolic acidosis on the fixed-acid Bohr effect in snapper (*Pagrus auratus*) following angling stress. *Comaprative Biochemistry and Physiology Part A* **154**, 56-60

Wells, R.M.G. and Weber, R.E. (1990). The spleen in hypoxic and exercised rainbow trout. *Journal of Experimental Biology* **150**, 461-466

West, G.B. and Brown, J.H. (2005). The origin of allometric scaling laws in biology from genomes to ecosystems: towards a quantitative unifying theory of biological structure and organisation. *Journal of Experimental Biology* **208**, 1575-1592

West, G.B., Brown, J.H. and Enquist, B.J. (1999). The fourth dimension of life: fractal geometry and allometric scaling of organisms. *Science* **284**, 1677-1679

Westerblad, H., Allen, D.G. and Lännergren, J. (2002). Muscle fatigue: lactic acid or inorganic phosphate the major cause? *News in Physiological Science* **17**, 17-21

White, C.R., Phillips, N.F. and Seymour, R.S. (2006). The scaling and temperature dependence of vertebrate metabolism. *Biology Letters* **2**, 125-127

Wieser, W. (1973). Temperature relations of ectotherms: a speculative review. In *Effects of Temperature on Ectothermic Organisms*. (ed. Wieser, W.) Springer Verlag, Berlin. pp. 1-23

Wieser, W. (1985). Developmental and metabolic constraints of the scope for activity in young rainbow trout (*Salmo gairdneri*). *Journal of Experimental Biology* **118**, 133-142

Wilkie, M.P., Brobbel, M.A., Davidson, K., Forsyth, L. and Tufts, B.L. (1997). Influences of temperature upon the postexercise physiology of Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Science* **54**, 503-511

Wilson, R.W. and Egginton, S. (1994). Assessment of maximum sustainable swimming performance in rainbow trout (*Oncorhynchus mykiss*). *Journal of Experimental Biology* **192**, 299-305

Winger, P.D., He, P. and Walsh, S.J. (1999). Swimming endurance of American plaice (*Hippoglossoides platessoides*) and its role in fish capture. *ICES Journal of Marine Science* **56**, 252-265

Winger, P.D., Eayrs, S. and Glass, C.W. (2010). Fish behaviour near bottom trawls. In *Behaviour of Marine Fishes: Capture Processes and Conservation Challenges*. (ed. He, P.). Wiley-Blackwell, Ames, Iowa. pp. 67-103

Witthames, P.R. and Greer Walker, M. (1982). The activity of myofibrillar and actomyosin ATPase in the skeletal muscle of some marine teleosts in relation to their age and length. *Journal of Fish Biology* **20**, 471-478

Wood, C.M. (1991). Acid-base and ion balance, metabolism and their interactions, after exhaustive exercise in fish. *Journal of Experimental Biology* **160**, 285-308

Wood, C.P. and Perry, S.F. (1985). Respiratory, circulatory and metabolic adjustments to exercise in fish. In *Circulation, Respiration and Metabolism. Current Comparative Approaches*. (ed. Gilles, R.). Springer-Verlag, Berlin. pp. 2-22.

Wood, C.M., Turner, J.D. and Graham, M.S. (1983). Why do fish die after severe exercise? *Journal of Fish Biology* **22,** 189-201

Woodward, C. and Strange, R.J. (1987). Physiological stress responses in wild and hatchery-reared rainbow trout. *Transactions of the American Fisheries Society* **116**, 574-579

Worm, B., Hilbourn, R., Baum, J.K., Branch, T.A., Collie, J.S., Costello, C., Fogarty, M.J., Fulton, E.A., Hutchings, J.A., Jennings, S., Jensen, O.P., Lotze, H.K., Mace, P.M., McClanahan, T.R., Minto, C., Palumbi, S.R., Parma, A.M., Ricard, D., Rosenberg, A.A., Watson, R. and Zeller, D. (2009). Rebuilding global fisheries. *Science* **325**, 578-585

Wu, R.S.S. (2002). Hypoxia: from molecular responses to ecosystem responses. *Marine Pollution Bulletin* **45**, 35-45

Wu, R.S.S. (2009). The effects of hypoxia on fish reproduction and development. In *Fish Physiology, Vol. 27. Hypoxia*.. (eds. Richards, J.G., Farrell, A.P. and Brauner, C.J.). Elsevier, London. pp. 79-141

Xiaojun, X. and Ruyung, S. (1990). The bioenergetics of the southern catfish (*Silurus meridionalis* Chen). I. Resting metabolic rate as a function of body weight and temperature. *Physiological Zoology* **63**, 1181-1195

Yamamoto, K. (1987). Contraction of the spleen in exercised cyprinid. *Comparative Biochemistry and Physiology* **87A**, 1083-1087

Yamamoto, K. (1988). Contraction of the spleen in exercised freshwater teleost. *Comparative Biochemistry and Physiology* **89A**, 65-66

Yamamoto, K., Itazawa, Y. and Koboyasju, H. (1980). Supply of erythrocytes in the circulating blood from the spleen of exercised fish. *Comparative Biochemistry and Physiology* **65A**, 5-11

Yamamoto, K., Itazawa, Y. and Koboyashi, H. (1983). Erythrocyte supply from the spleen and haemoconcentration in hypoxic yellowtail. *Marine Biology* **73**, 221-226

Zabel R.W., Harvey C.J., and Katz S.L. (2003). Ecologically sustainable yield: Marine conservation requires a new ecosystem-based concept for fisheries management that looks beyond sustainable yield for individual species. *American Scientist* **91**, 150-157

Zhang, W., Cao, Z.D., Peng, J.L., Chen, B.J. and Fu, S.J. (2010). The effects of dissolved oxygen level on the metabolic interaction between digestion and locomotion in juvenile southern catfish (*Silurus meridionalis* Chen). *Comparative Biochemistry and Physiology, Part A* **157**, 212-219

APPENDIX ONE

Seasonal variation in water temperature at Nelson Haven

The geography of Nelson Haven, firstly as an area of relatively shallow, tidal flats, and secondly, within the greater Tasman Bay area, is such that it experiences substantial seasonal variation in temperature. The New Zealand Institute for Plant and Food Research facility is located on the waterfront of Nelson Haven, and water is drawn directly from the Haven for use in the tank facilities. Fish maintained within these facilities are therefore exposed to these large seasonal temperature gradients.

The temperature of the incurrent water drawn from the Haven from the period 1 January to 31 December 2012 is presented in Figure A1.1. Minimum and maximum temperatures during this period were 9.9 and 21.6 ^{o}C, respectively. The mean annual temperature was 15.1 $\pm$ 0.2 ^{o}C.

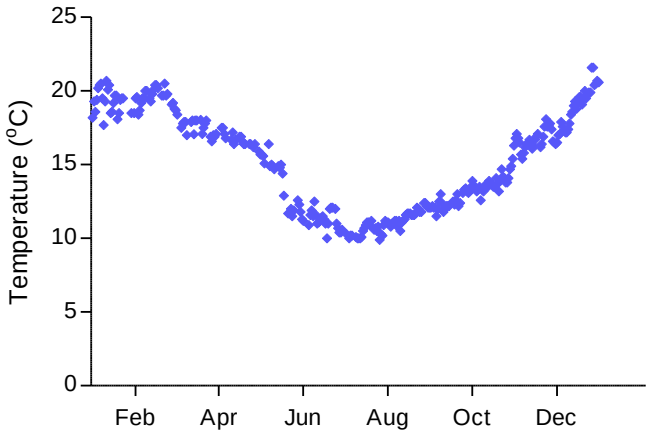


Figure A1.1. Daily record of water temperature in Nelson Haven, for the period 1 January to 31 December 2012.

APPENDIX TWO

Recovery of MO_2 following anaesthesia

Introduction

The following preliminary experiment was carried out to determine whether 18 hours represented a sufficient period of time for fish to recover from the stresses associated with handling, anaesthesia and adjustment to the confines of the respirometer, prior to the recording of $MO_{2\ routine}$.. In addition, some species exhibit diurnal fluctuation in MO_2 that may complicate the measurement of MO_2 (Steffensen, 1989), hence we sought to determine whether this was the case for snapper, or more specifically, whether this was the case under a 24 h light : 0 h dark lighting regime.

Methods

The experiment was performed using the experimental setup and methodologies detailed in Section 2.4. Temperatures were maintained at 18 $\pm$ 0.2 ^{o}C, as to be consistent with the temperature used throughout the majority of experiments in this thesis.

Snapper (64.3 $\pm$ 5.3g; n = 8) were anaesthetised in 50 mg l^{-1} MS-222 for approximately 1 minute, until swimming movements ceased. They were then weighed and measured, and placed into individual respirometers. Each respirometer was then sealed, and the automated Fast Response Output feature of Chart v7.3 was used to facilitate the recording of PO_2 under normoxic conditions (120-140 mmHg) over the subsequent 24 hour period.

Results

Snapper rapidly regained equilibrium when replaced into fresh seawater. Fish would often show distress at being confined within the respirometer, with the degree of agitation being variable between individuals. MO_2 decayed rapidly during the first 4 hours of recovery and appeared to stabilise thereafter, such that the mean MO_2 for the period 4 to 6 hours post-anaesthesia was not significantly different to that at 16-18 or 22-24 hours post-anaesthesia ($p = 0.1738$ and $p = 0.2279$, respectively; Fig. A2.1). Two of the fish were monitored for a total of 42 hours following anaesthesia; although MO_2 was slightly (~9%) lower at 40-42 than 22-24 hours post-anaesthesia, a one-way ANOVA revealed no significant differences between MO_2 at 16-18, 22-24 and 40-42 hours ($p = 0.2278$).

Together, the data indicate that a recovery period of 18 hours following introduction to the respirometer is sufficient for the recovery of MO_2 to stable levels appropriate for the determination of $MO_{2\ routine}$. Further, there was no evidence of diurnal fluctuations in MO_2, possibly as a result of the 24 h light : 0 h dark lighting regime implemented within the experimental room.

Recovery from AQUI-S

The following data represent that from which $MO_{2\ routine}$ was calculated for 550 g fish during the study reported in Chapter 3. Time constraints associated with working in Nelson did not permit a preliminary set of experiments for the determination of appropriate recovery times; rather, following the instalment of snapper into the respirometer, MO_2 was monitored for the following 24 hours, such that recovery rates could be determined and $MO_{2\ routine}$ subsequently calculated at an appropriate time.

Snapper (542 $\pm$ 21 g, n = 8) were anaesthetised in 20 ppm AQUI-S^{TM} for 30 minutes, then weighed and measured, and placed into the flow-through respirometry system described in Section 2.4.3. The flow-though system ensured normoxic conditions were maintained over the 24-hour period during which MO_2 was monitored.

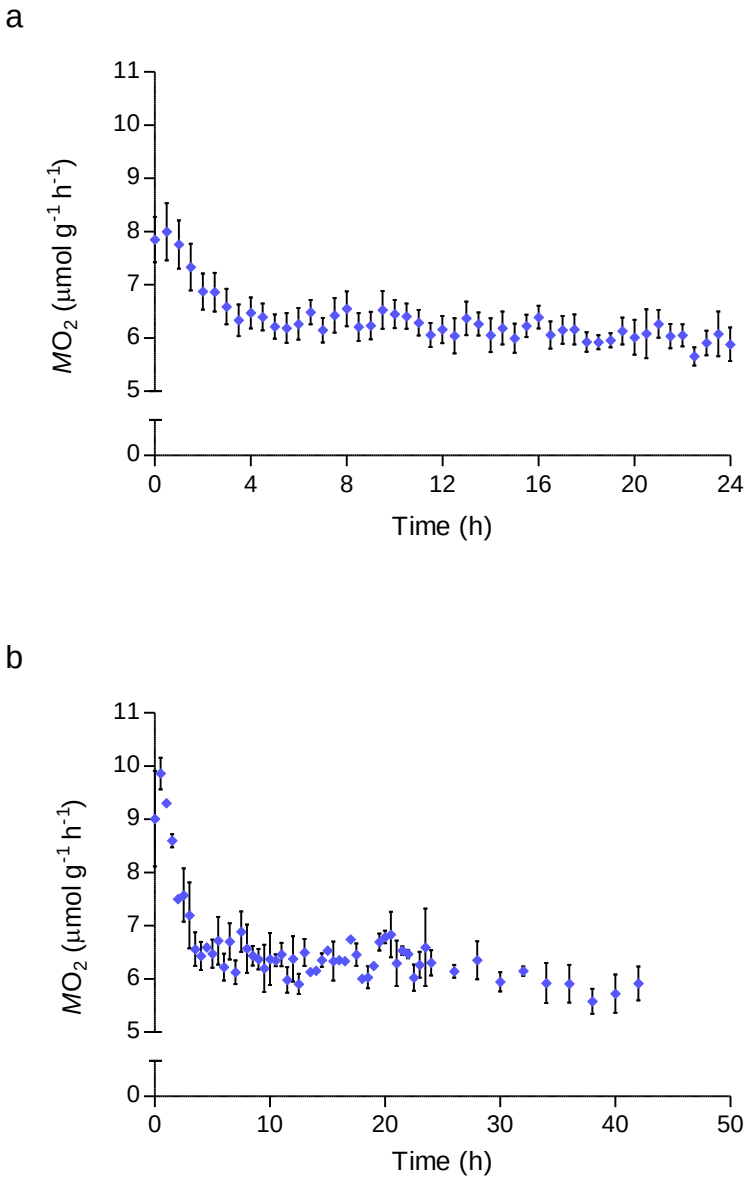


Figure A2.1. Oxygen consumption in snapper following anaesthesia in 50 mg l^{-1} MS-222 and subsequent placement into a closed-box respirometry system (a, n = 8; b, n = 2).

Fish behaviour within the respirometer was monitored via the webcam; snapper were observed to become quite distressed as they initially recovered from anaesthesia, likely due to the restrictive dimensions of the respirometer that prevented them from turning 180°. Following peak MO_2 approximately 1 hour after placement into the respirometer, MO_2 decayed to an apparent plateau at approximately 8 to 12 hours post-anaesthesia (Fig. A2.2). The elevation of MO_2 at approximately 17 hours is attributable to the arrival of people at work and their movement around the small laboratory space the following morning. This highlights the importance of having respirometry work performed in an appropriately quiet and controlled environment. $MO_{2\ routine}$ was therefore determined for 550 g snapper as being the mean MO_2 for each fish between 10 and 12 hours post-anaesthesia.

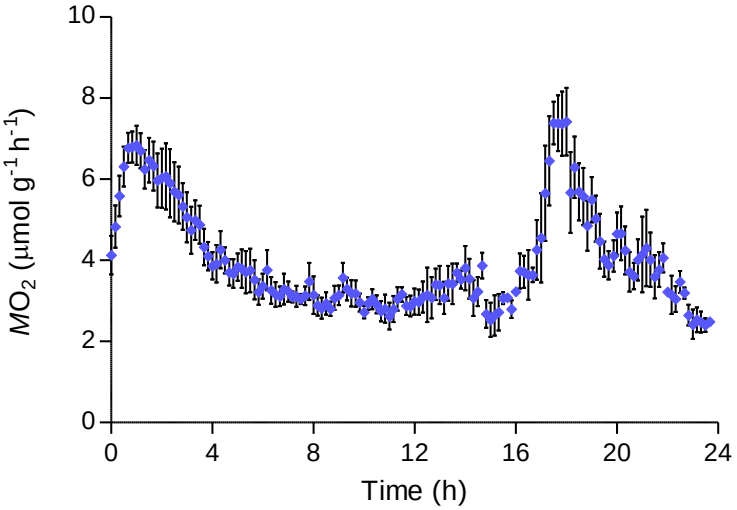


Figure A2.2. Oxygen consumption in snapper following anaesthesia in 20 ppm AQUI-S™ and subsequent placement into a flow-through respirometry system (n=8).

APPENDIX THREE

Examination of methodology for the sampling and processing of white muscle samples for lactate and glycogen

Introduction

In an attempt to discern the reason(s) for the metabolic status of the white muscle (WM) samples taken from snapper pre-exercise (Chapter 5), where endogenous glycogen reserves appeared to have been metabolised to lactate, a further set of experiments was performed to determine whether elements of the collection and/or processing of samples during analysis could have contributed to the metabolic status of the tissue. In particular, the efficiency of tissue extraction and the stability of the metabolites are important to accurately determining metabolic status; incomplete degradation of cell membranes could compromise the release of metabolites to the medium, whilst the incomplete denaturation of enzymes and/or warming of tissue during extraction could permit continued glycolytic activity, depleting metabolic substrates (Wang et al., 1994). We therefore sought to determine the efficiency of tissue extraction by comparing metabolite concentrations obtained from tissues homogenised using the Ultra-Turrax, with those of tissues ground under liquid nitrogen in a pre-cooled mortar and pestle. The possible lability of glycogen and lactate to any thaw of the tissue during processing was investigated by comparing metabolite concentrations in samples that had been allowed to thaw completely, with those of tissue that remained completely frozen. In addition, we sought to determine whether anaerobic processes were induced by perfusion limitation of tissues following euthanasia and blood sampling; the tendency of the blood to clot meant the preparation of blood samples for analysis was prioritised, prior to the excision of the muscle and liver samples – a delay of approximately 5 minutes. A time series of muscle sampling (0 to 30 minutes post-mortem) was established during the present work to

determine whether this delay could have impacted metabolite concentrations. Finally, to determine whether the metabolic depletion within the WM was attributable to confinement of fish within the recovery system, we sought to compare the metabolic status of fish sampled from the recovery tanks, with those sampled directly from the aquarium itself. These comparisons are summarised in Figure A3.1.

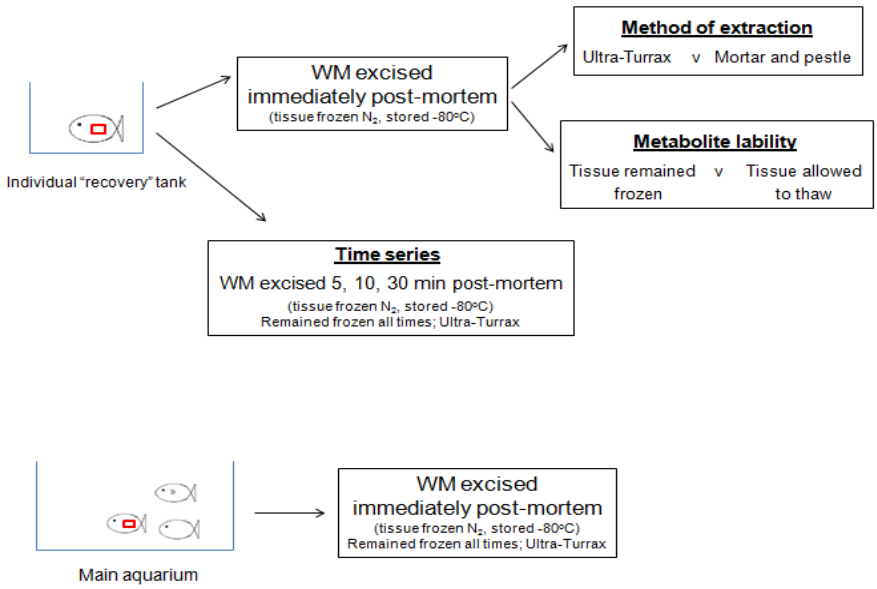


Figure A3.1. Summary of the different sampling techniques used in an attempt to discern the possible reason(s) for the metabolic status of the WM samples obtained during experimental work presented in Chapter 5.

Methods

Four snapper (153.6 ± 5.2 g) were sampled as rested fish, as described in Chapter 5. Fish were carefully netted and transferred to individual 30 l tanks housed within a controlled environment facility, in which the air temperature was maintained at 18 ºC, and with a lighting regime of 24 hours light : 0 hours dark. After 24 hours, a concentrated solution of

MS-222 was added to the tank to give a final concentration of 150 mg l^{-1}. Following the cessation of all movement (approximately 2 minutes), fish were removed from the tank and killed by spinal cord ablation. No blood samples were taken during this study; the focus was simply to determine the effects of subtly different sampling techniques on muscle metabolite status. Immediately post-mortem, one side of the fish was gently de-scaled, and a small WM sample excised, from which the skin and superficial muscle layer were removed. The sample was subsequently split into several subsamples which were rapidly frozen in liquid nitrogen. Additional WM samples were excised and frozen at 5, 10 and 30 minutes post-mortem. All samples were subsequently stored at -80 ^{o}C until analysis. Samples were analysed within 72 hours.

Series I – Time series of tissue extraction

For each fish, WM samples extracted immediately (i.e. 0 min) and 5, 10 and 30 minutes post-mortem were assayed for lactate and glycogen as described in Chapter 5. Briefly, for the determination of lactate, approximately 200 mg of tissue was homogenised on ice in 6% PCA using an Ultra-Turrax. The homogenate was centrifuged and the supernatant neutralised with 3M $KHCO_3$, then centrifuged again. Lactate concentration within the supernatant was determined using an ABL-725 analyser. For the determination of muscle glycogen concentration, approximately 200 mg of WM was digested in 30% KOH at 80 ^{o}C for 20 minutes, and following a series of rinses by precipitating glycogen in ethanol and redissolving it in distilled water, a subsample of the supernatant was incubated with anthrone reagent at 80 ^{o}C for 15 minutes. Glycogen concentration was determined by measuring absorbance at 620 nm and calculating against a standard curve.

Metabolite concentrations at the different excision times were compared using repeated-measures ANOVA.

Series II – Comparison of extraction method

To verify the efficiency of extraction technique, one of the samples excised immediately from each fish was ground to a fine powder under liquid nitrogen using a pre-cooled mortar and pestle. The powder was then transferred using a pre-cooled spatula to an eppendorf tube

containing a pre-measured volume of ice-cold 6% PCA and assayed as above. The lactate concentration within the sample was compared with that of the equivalent sample from Series I (i.e. another 0 min sample from the same fish, homogenised using Ultra-Turrax) using a paired t-test.

Series III – Metabolite lablity

To determine the lability of lactate and glycogen to any thawing of the muscle samples, one of the samples excised immediately from each fish was allowed to thaw completely (1 hour at room temperature) prior to analysis. The tissues were subsequently assayed for lactate and glycogen as described in Series I. Metabolite concentrations were compared with those of equivalent samples (0 min, same fish) from Series I, where the tissue had remained frozen during processing, using a paired t-test.

Series IV – Metabolic status of fish within the main aquarium

To determine whether the metabolic status of the WM was associated with some element of the confinement of fish within the recovery tanks, two snapper (~150 g) were sampled directly from the main aquarium system. Fish were rapidly caught with a dip-net and killed with an acute blow to the head, taking approximately 3-5 seconds. Fish were then gently de-scaled and WM samples excised and frozen in liquid nitrogen as described above. The total time from the netting of the fish to the freezing of the tissues was <2 minutes. Samples were subsequently assayed for lactate and glycogen as described in Series I, and metabolite concentrations compared with the 0 min samples from Series I, using a Student's t-test of unpaired design.

For all statistical analysis, significance was taken at the level $p < 0.05$.

Results and Discussion

Although WM samples ground in the mortar and pestle had a tendency towards lower lactate levels than did samples homogenised in the Ultra-Turrax, there were no significant differences in either muscle lactate or glycogen as a result of the different preparation techniques (all $p > 0.05$; Fig. A3.2). Further, there was no significant difference between either muscle lactate ($p = 0.8648$) or glycogen ($p = 0.5623$) concentration in fish sampled from the recovery system and those sampled directly from the main aquarium system. In all cases, WM lactate concentration was in excess of 25 μmol g^{-1} and glycogen concentration less than 2 μmol g^{-1} – consistent with those observed during the experimental work of Chapter 5.

The reason for the apparent depletion of glycolytic reserves remains unresolved. The fact that WM samples that were excised immediately post-mortem, promptly frozen in liquid nitrogen, stored at -80 ^{o}C and subsequently processed under liquid nitrogen, exhibit these high lactate and low glycogen levels suggests these metabolite concentrations are present prior to the excision and freezing of the tissue. As discussed in Chapter 5, the involvement of acute confinement- or hypoxia-related stresses within individual recovery tanks is not supported by the haematological data, or plasma lactate or cortisol levels. The high lactate, low glycogen content of the WM of fish sampled directly from the main aquarium further suggests that the metabolic depletion is not attributable to the recovery tank system.

Given the relative lactate and glycogen concentrations of the WM of fish sampled from the aquarium, there remains several possible explanations for the observed results. Firstly, that these fish may exist with inherently high lactate and low glycogen levels within the WM. Secondly, the relative concentrations of lactate and glycogen could reflect the rapid mobilisation of energy reserves in response to an acute stressor immediately prior to death, for example, the introduction of MS-222 to the system or acute struggling within the dip-net. Finally, it is possible that a methodological error, which facilitated continued glycolytic activity has been overlooked. Each of these possibilities has its own reservations, and these are discussed in Chapter 5, in the context of the other physiological data obtained during the main experimental work.

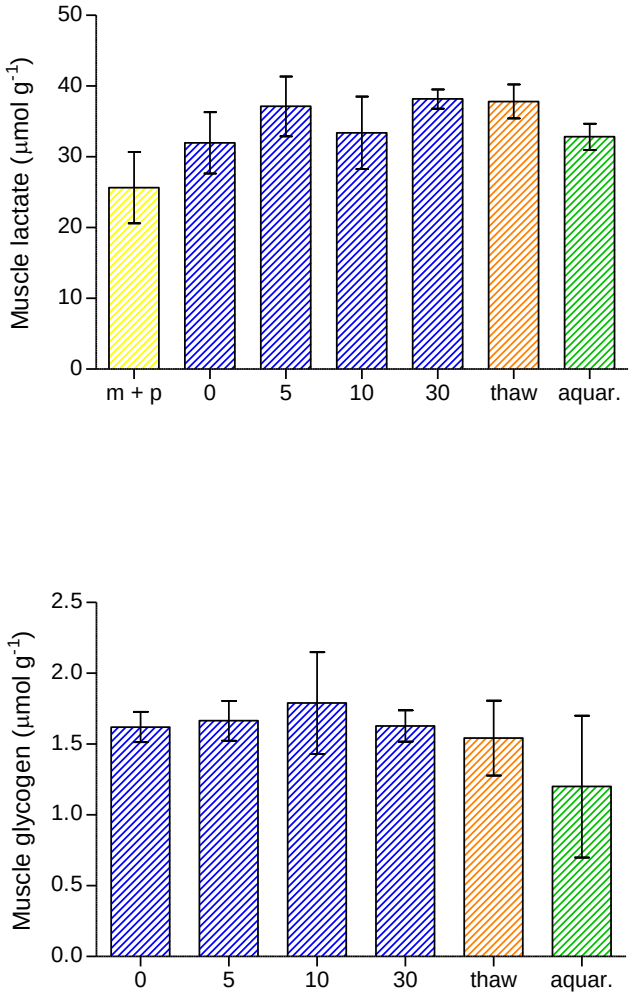


Fig. A3.2. Lactate and glycogen concentration in the white muscle of snapper, as determined using different sample preparation techniques. m + p represents those samples processed by grinding tissue to a fine powder in a mortar and pestle; 0, 5, 10 and 30 represent the time-series of samples, denoting the time (in minutes) post-mortem that samples were excised, with subsequent homogenisation using the Ultra-Turrax prior to assay; thaw refers to those samples obtained at time 0, but allowed to thaw completely (1 hour at room temperature) prior to homogenisation using the Ultra-Turrax and subsequent assay; aquar. denotes samples obtained from snapper sampled directly from the aquarium.